THE PHILIPPINE
JOURNAL OF SCIENCE

VOL. 51 AUGUST, 1933 No. 4

DURATION OF THE SEROLOGIC REACTIONS IN MONKEYS INOCULATED WITH YAWS OR SYPHILIS

By ONOFRE GARCIA

Of the Division of Biology and Serum Laboratory, Bureau of Science, Manila

It has been determined in previous experiments that the serologic picture in experimental yaws and syphilis[1] consists of two phases. The early serologic reaction coincides with the formation of a primary lesion and disappears at the time when this heals. The late serologic response has been demonstrated in animals that developed only primary lesions; it appeared about six months after inoculation and coincided with the resistance to superinoculation.[2] The effect of specific treatment on the early serologic response has also been ascertained. Animals that gave positive results before treatment were repeatedly examined and found negative.[3]

The duration of the early as well as the late serologic reaction has been studied in this laboratory up to a certain limit of time. The present investigation is concerned with the study of the duration of the late serologic reaction beyond the time limit previously tested. It also shows the result of experiment on the effect of specific treatment with neosalvarsan on the late serologic reaction.

PAST HISTORY OF THE ANIMALS USED

The monkeys employed in this experiment represent groups of animals that were used for the study of immunity conferred by either single infection or reënforced by vaccination or super-

[1] Philip. Journ. Sci. 42 (1930) 205, 206, and 210.
[2] Philip. Journ. Sci. 42 (1930) 211.
[3] Philip. Journ. Sci. 35 (1928) 292.

inoculation. After being treated with neosalvarsan some of these animals were reinoculated with yaws alone or with both yaws and syphilis. The history of each individual monkey may be consulted in the accompanying tables. Tables 1, 2, and 3 show the dates, number, and kind of inoculations received by each monkey, while Tables 4, 5, and 6 show the period of time that elapsed between the first inoculation and the last serologic tests, Wassermann [4] and Kahn,[5] performed on each animal. Table 7 shows the group of animals that were treated at the early and late stages of infection.

DISCUSSION OF RESULTS

As may be seen from the results of the Wassermann and Kahn tests given in Tables 1, 2, and 3, the animals, with very few exceptions, showed a rather strong serologic response. The Kahn test, which was not repeated, was negative with monkeys O–c–1, O–C–2, L–13, Sy–G–22, and Sy–P–23. However, the results of the Wassermann test with the same animals were markedly positive. The animals did not show any external lesions at the time of collecting blood.

Variations in the strength of the serologic reactions were found in the treated animals, in some of which the serologic reaction was either stationary, very low, or very sluggish before it became entirely negative.[6] The animals J–11, T–4, W–25, and W–27, which received treatment during their high resistance—that is, late after inoculation—continued to show rather strong serologic reactions at the time of examination, while in monkeys K–25, K–26, K–27, and K–28, which were treated in the early stage of infection, the degree of serologic reactions was entirely individual. Besides, a degree of sensitization was more profound in the first group than in the latter group of monkeys (see Table 7). This finding agrees with clinical experience, which has shown that the effect of treatment upon serologic reactions is more evident when such treatment is given in an early stage of syphilis than when given in a late stage of infection. It was also ascertained in previous experiments [7] that the Wassermann reaction, if it has become negative due to treatment or spontaneous healing, and if all the lesions have disappeared,

[4] Philip. Journ. Sci. § B 12 (1917) 249.
[5] The Kahn Test, a Practical Guide. Williams and Wilkins Co. (1928).
[6] Philip. Journ. Sci. 35 (1928) 311.
[7] Philip. Journ. Sci. 35 (1928) 261; 40 (1929) 53.

will reappear upon unsuccessful superinfection or reinoculation with killed or viable material.[8]

CONCLUSION

The results obtained in the late serologic reactions of the experimental monkeys studied herewith confirm the views regarding sensitization caused by the inoculations of killed or living treponema. The strength of the serologic reaction depends on the degree of sensitization of the tissue as influenced by the extension and duration of lesions or by frequent inoculations. The degree of the serologic reaction, however, is modified by specific treatment.

ACKNOWLEDGMENT

I wish to express my sincere appreciation to my chief, Dr. Otto Schöbl, for supplying materials for study.

[8] Philip. Journ. Sci. 35 (1928) 291–311; 42 (1930) 203–211.

TABLE 1.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with syphilis and yaws.*

Monkey.	Syphilis.			Lymph-gland transplant.		Yaws.		
	Date.	Site of inoculation.	Result.	Date.	Result.	Date.	Site of inoculation.	Result.
J–11	IX–13–28	Intradermal	+	XI–16–28	—	III–19–26	Intradermal	+
						VII– 6–26	do	+
						IX–29–26	do	—
						IV– 4–27	do	—
						III–26–28	do	—
J–11	I– 7–30	Test for immunity	—			I–11–29	do	—
T–4	IX–13–28	Intradermal	—	XI–16–28	—	VII– 2–26	Intradermal	+
						VIII–20–26	do	+
						III– 8–27	do	
						III–26–28	do	—
T–4	I– 7–30	Test for immunity	—			I– 8–29	do	—
U–1						VII–27–27	Subcutaneous yaws 60° C.	
						VII–30–27		
						VIII– 3–27		
U–1						IX–17–27	Test for immunity to yaws	—
						III– 9–28		
						IV–23–28		
U–1	I– 7–29	Intradermal immunity test	—	0	0	II–14–30	Last test for immunity to yaws	—
W–25						I–20–28	Subcutaneous yaws 60° C.	
						I–31–28	do	
W–25						II–27–28	Test for immunity to yaws	—
						V– 3–28		
						VI–25–28		

W–25	I– 7–29	Intradermal immunity test	—	IV–26–29	+	IX–17–28	Reinfection	—
						X–22–28	do	—
						I– 7–29	do	—
						II–14–30	Immunity test	—
W–23						I–18–28 I–31–28 II– 8–28	Subcutaneous yaws 60° C.	
W–23						II–27–28 IV–23–28 VI– 4–28	Test for immunity to yaws	—
W–23	I– 7–29	Intradermal immunity test	—	V–13–29	+	IX–17–28	Reinfection	—
						X–22–28	do	
						I– 7–29	do	—
						II–14–30	Immunity test	—
W–27						I–20–28 I–31–28	Subcutaneous yaws 60° C.	
W–27						II–27–28 V– 3–28 VI–25–28	Test for immunity to yaws	—
W–27	I– 7–29	Intradermal immunity test	—	0	0	IX–17–28	Reinfection	—
						X–22–28	do	—
						I– 7–29	do	—
						II–14–30	Immunity test	—
E–41	VI–22–29	Intradermal	—	IX–11–29	—	II– 1–28	Vaccination 60° C.	
						II–28–28	Intradermal	+
						II–27–29	Superinoculation	—
Sy–3	VI–13–28	Intradermal	+	IX– 8–28	+	IX–11–28	Intradermal	+
	I– 7–30	Immunity test	—					
Sy–5	VI–15–28	Intradermal	+	X–22–28	+	XI–13–28	do	+
	I– 7–30	Immunity test	—					
J–1	VI–22–29	Intradermal	—	IX–26–29	—	VIII–17–28	Intradermal	+
						II–27–29	Test for immunity	—

TABLE 1.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with syphilis and yaws*—Continued.

Monkey.	Syphilis.			Lymph-gland transplant.		Yaws.		
	Date.	Site of inoculation.	Result.	Date.	Result.	Date.	Site of inoculation.	Result.
Sy–G–22	VI–22–29	Intradermal	+	X– 4–29	+	X–14–29	Intradermal	+
	VIII–14–29	do	—			X–21–29	Superinfection	+
	IX–27–29	Test for immunity	—			XII– 2–29	do	+
						I– 6–30	do	+
						II–26–30	do	—
Sy–G–20	II– 5–29	Intradermal	+					
Sy–G–20	V–29–29	do	—			X– 8–29	Intradermal	—
	IX–27–29	Test for immunity	—			XII– 2–29	Superinfection	—
Sy–J–20	II– 9–29	Intradermal	+			X– 8–29	Intradermal	—
						XII– 2–29	do	—
Sy–J–20	I– 7–30	Test for immunity	—					
Sy–I–11	III–20–29	Intradermal	+			IX–21–29	Intradermal	+
	VIII–14–29	do	—			X–21–29	do	+
Sy–I–11						I– 6–30	Superinfection	—
						II–26–30	do	—
						III–21–29	Intradermal	+
O–c–1	V–27–29	do	+			VII–17–29	Superinoculation	—
						VIII–16–29	do	—
						III–21–29	Intradermal	+
O–c–2	V–27–29	do	+			VII–17–29	Superinoculation	—
						VIII–16–29	do	—
						III– 4–29	Intradermal	+
L–13	V–27–29	do	+			VII–17–29	Superinoculation	—
						VIII–16–29	do	—

Sy–P–23	VIII–14–29	do	+	X–14–29	Intradermal	+
	IX–27–29	do	–	X–21–29	do	+
				I– 6–30	Superinfection	+
Yac–10	IX	do	–	II–26–30		–
				VII– 9–29	Intradermal	+
				VIII–28–29	Superinfection	–
K–25	II–13–30	Vaccination 60° C		XI–27–29	Intradermal	+
	II–25–30			III–27–30	do	–
	III– 4–30			V–20–30	do	–
K–26	II–13–30	do		XI–27–29	Intradermal	+
	II–25–30	do		III–27–30	do	–
	III– 8–30			V–20–30	do	–

TABLE 1.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with syphilis and yaws*—Continued.

Monkey.	Neosalvarsan treatment.		Last test for Wassermann and Kahn reactions.			Test for duration.					
						Wassermann.				Kahn.	
	Date.	Dose.	Date.	Wassermann (average).	Kahn (average).	Date.	Alcohol.	Control.	Cholest.	0.025	0.0125
		g.									
J–11	XII– 2–26	0.09									
J–11						VIII– 6–30	++++	—	++++	+	++++
T–4	I–25–27 to XII–14–27	0.155									
T–4						X–25–30	++++	—	++++	++	++
U–1											
U–1											
U–1						VI–27–30	+++	—	++++	++++	++++
W–25				+							
W–25	VIII– 3–28 to XII–14–28	0.18		++++							
W–25		0.01				VI–27–30	++++	—	++++	++++	++++
W–23				+							
				—							
W–23	VIII– 3–28	0.18		+++							
W–23	XII–14–28	0.01				VI–27–30	±	—	++++	+++	++++
W–27				+							
W–27	VIII– 3–28	0.18		++++							
W–27	XII–14–28	0.01				VI–27–30	—	—	+++	++++	++++

			4th week	—						
E–41			After infected.	+		VII– 7–30	++++	—	++++	++++	++++
Sy–3			The Wassermann and Kahn reactions were irregular up to the 57th week, becoming strongly 4+ up to the 66th week and the 69th week.			VI–24–30	—	—	++++	—	+++
Sy–5						VI–24–30	+	—	++++	++	++++
J–1						VIII– 6–30	++++	—	++++	++	++++
Sy–G–22						VII–11–30	—	—	+	—	—
Sy–G–20			Wassermann and Kahn were negative during 39 weeks' observation.								
Sy–G–20						VII–11–30	++	—	++++	++++	++++
Sy–J–20						VII–16–30	±	—	++++	—	++
Sy–I–11											
Sy–I–11						VII–11–30	±	—	++++	+	++++
O–c–1						VII–25–30	++++	—	++++	—	—
O–c–2						VII–25–30	—	—	++++	—	—
L–13						VII–23–30	±	—	++++	—	—
Sy–P–23						VII–25–30	++++	—	++++	—	—
Yac–10						VII– 1–30	—	—	++++	+	++++
K–25	I–21–30 I–27–30	0.03	6th week	+	—	VIII– 9–30	—	—	±	—	—
			8th week	++	+						
			11th week	±	—						
			14th week	±	—						
			16th week	++	—						
K–26	I–21–30 I–27–30	0.03	6th week	+	—	VIII– 9–30	—	—	±	—	—
			8th week	++	+						
			11th week	+	—						
			14th week	++++	—						
			16th week	+++	—						

TABLE 2.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with yaws alone.*

Monkey.	Syphilis.			Lymph-gland transplant.		Yaws.		
	Date.	Site of inoculation.	Result.	Date.	Result.	Date.	Site of inoculation.	Result.
J–18						IV–16–28	Vaccination 100° C.	
						VI–26–28	Intradermal	+
						II–27–29	Superinoculation	—
f–2						VI–25–28	Intradermal	+
						II–27–29	Superinoculation	—
Monkey No. 1, Guzon strain						X–15–28	Intradermal	+
						I–26–29	Autosuperinoculation on both eyebrows	?
Z–1							Inflammatory skin tissues from yaws monkeys	
						VIII– 2–28	Intradermal	Yaw
L–15						IV– 8–29		
						VI–18–29	Superinoculation	—
						VII–11–29	do	—
Y–G–25						X– 8–29	Intradermal	+
K–12						V–10–29	do	—
						VII–11–29	do	—
K–13						V–10–29	do	+
F–38						VI–29–29	do	+
YB–9						VII–17–29	do	+
						VIII–16–29	do	+
YM–20						IX–21–29	do	+
						X–21–29	do	+
K–27						XI–27–29	do	?
						III–27–30	do	+
K–28						XI–27–29	do	+
						III–27–30	do	+

TABLE 2.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with yaws alone*—Continued.

Monkey.	Neosalvarsan treatment.		Last test for Wassermann and Kahn reactions.			Test for duration.					
	Date.	Doses.	Date.	Wassermann (average).	Kahn (average).	Date.	Wassermann.			Kahn.	
							Alcohol.	Control.	Cholest.	0.025	0.0125
J–18			V–17–28	++++	+++						
						X–25–30	++++	—	++++	+++	++
f–2						VIII– 6–30	++++	—	++++	++++	++++
Monkey No. 1, Guzon strain						XI– 7–30	—	—	—	—	—
Z–1			VI–20–28	—							
			VII– 5–28	—							
			IX– 3–28	—							
			IX–13–28	—							
			IX–27–28	—							
			X– 9–28	—							
			X–30–28	—							
			XI–14–28	±							
			XII– 7–28	—							
						V–25–30	++++	—	++++	++	++
I–15						VII–23–30	+++	—	++++	+++	++++
Y–G–25						VIII– 8–30	++++	—	++++	++++	++++
K–12			VIII–12–29	Microscopically positive for spirochete.		VII–16–30	+	—	++++	++	++++
K–13						VII–16–30	++++	—	++++	—	++++
F–38						VII– 7–30	—	—	++++	—	++
YB–9						VII– 1–30	++++	—	++++	++++	++++
YM–20						VII–23–30	—	—	++++	—	++++

TABLE 2.—*Showing the duration of Wassermann and Kahn tests in untreated and treated monkeys injected with yaws alone*—Continued.

Monkey.	Neosalvarsan treatment.		Last test for Wassermann and Kahn reactions.			Test for duration					
	Date.	Doses.	Date.	Wassermann (average).	Kahn (average).	Date.	Wassermann.			Kahn.	
							Alcohol.	Control.	Cholest.	0.025	0.0125
K–27	I–21–30 I–27–30	0.03	6th week	++	±						
			8th week	++++	+						
			11th week	—	—						
			14th week	+++	—						
			16th week	+	—	VIII– 9–30	—	—	+	—	—
K–28	I–21–30 I–27–30	0.03	6th week	+	—						
			8th week	+++	++						
			11th week	±	—						
			14th week	++	—						
			16th week	+++	—	VIII– 9–30	—	—	++++	—	++++

TABLE 3.—*Showing the duration of Wassermann and Kahn tests in untreated monkeys injected with syphilis alone.*

Monkey.	Syphilis.			Lymph-gland transplant.		Yaws.		
	Date.	Site of inoculation.	Result.	Date.	Result.	Date.	Site of inoculation.	Result.
Sy-D-20	V-27-29	Intradermal	+					
Sy-P-25	IX-27-29	do	+					

Monkey.	Neosalvarsan treatment.		Last test for Wassermann and Kahn.			Test for duration.					
	Date.	Doses.	Date.	Wassermann (average).	Kahn (average).	Date.	Wassermann.			Kahn.	
							Alcohol.	Control.	Cholest.	0.025	0.0125
Sy-D-20						VII-7-30	—	—	++++	—	++++
Sy-P-25						VIII-8-30	—	—	—	—	—

TABLE 4.—*Showing the duration of Wassermann and Kahn tests in untreated monkeys that were injected with both yaws and syphilis.*

Monkey.	Period between first injection and last test for duration of Wassermann and Kahn tests.		Number and kinds of inoculations.				Wassermann.			Kahn.	
			Alive.		Killed at 60° C.						
			Syphilis.	Yaws.	Syphilis vaccine.	Yaws vaccine.	Alcohol.	Control.	Cholest.	0.025	0.0125
	Yrs.	*mos.*									
U-1	3	0	1	4	0	3	++++	—	++++	++++	++++
E-41	2	5	1	2	0	1	++++	—	++++	++++	++++
Sy-3	2	0	2	1	0	0	—	—	++++	—	+++
Sy-5	2	0	2	1	0	0	+	—	++++	++	++++
J-1	1	9	1	2	0	0	++++	—	++++	++	++++
Sy-G-22	1	6	2	5	0	0	—	—	+	—	—
Sy-G-20	1	5	3	2	0	0	++	—	++++	++++	++++
Sy-J-20	1	5	2	2	0	0	±	—	++++	—	++
Sy-I-11	1	4	2	4	0	0	±	—	++++	+	++++
L-13	1	4	1	3	0	0	±	—	++++	—	—
O-c-1	1	4	1	3	0	0	++++	—	++++	—	—
O-c-2	1	4	1	3	0	0	—	—	++++	—	—
Sy-P-23	0	9	2	4	0	0	++++	—	++++	—	—
Yac-10	0	9	1	2	0	0	—	—	++++	±	++++

TABLE 5.—*Showing the duration of Wassermann and Kahn tests in untreated monkeys that were injected with yaws only.*

Monkey.	Period between first injection and last test for duration of Wassermann and Kahn tests.	Number and kinds of inoculations.				Wassermann.			Kahn.	
		Alive.		Killed at 60° C.						
		Syphilis.	Yaws.	Syphilis vaccine.	Yaws vaccine.	Alcohol.	Control.	Cholest.	0.025	0.0125
	Yrs. mos.									
J-18	2 4	0	2	0	1	++++	—	++++	+++	++
f-2	2 1	0	2	0	0	++++	—	++++	++++	++++
No. 1	2 0	0	2	0	0	—	—	—	—	—
Z-1	1 7	0	1	0	(a)	++++	—	++++	++	++
Y-G-25	1 3	0	1	0	0	++++	—	++++	++++	++++
L-15	1 3	0	3	0	0	+++	—	++++	++++	++++
K-12	1 2	0	2	0	0	+	—	++++	++	++++
K-13	1 2	0	1	0	0	++++	—	++++	—	++++
F-38	1 0	0	1	0	0	—	—	++++	—	++
YB-9	0 10	0	2	0	0	++++	—	++++	++++	++++
YM-20	0 8	0	2	0	0	—	—	++++	—	++++

[a] Tissue from yaws.

TABLE 6.—*Showing the duration of Wassermann and Kahn tests in untreated monkeys that were injected with syphilis only.*

Monkey.	Period between first injection and last test for duration of Wassermann and Kanh tests.	Number and kinds of inoculations.				Wassermann			Kahn.	
		Alive.		Killed at 60° C.						
		Syphilis.	Yaws.	Syphilis. vaccine.	Yaws vaccine.	Alcohol.	Control	Cholest.	0.025	0.0125
	Yr. mos.									
Sy-D-20	1 1	1	0	0	0	—	—	++++	—	++++
Sy-P-25	0 9	1	0	0	0	—	—	—	—	—

TABLE 7.—*Showing the duration of Wassermann and Kahn tests in treated monkeys that were inoculated twice or more with either yaws alone or both syphilis and yaws.*

Monkey.	Duration between first infection and last Wassermann and Kahn tests.	Number and kinds of inoculations.					Wassermann.			Kahn.	
		Syphilis.	Yaws.	Yaws vaccine.	Syphilis vaccine.	Neo-salvarsan treatment.	Alcohol.	Control.	Cholest.	0.025	0.0125
	Yrs. mos.										
J-11	4 5	2	6	0	0	0.09	++++	—	++++	+	++++
T-4	4 2	2	5	0	0	0.155	++++	—	++++	++	++
W-23	2 5	1	7	3	0	0.19	±	—	++++	+++	++++
W-25	2 5	1	7	2	0	0.19	++++	—	++++	++++	++++
W-27	2 5	1	7	2	0	0.19	—	—	++++	++++	++++
K-25	0 7	0	3	0	3	0.03	—	—	±	—	—
K-26	0 7	0	3	0	3	0.03	—	—	±	—	—
K-27	0 7	0	2	0	0	0.03	—	—	±	—	—
K-28	0 7	0	2	0	0	0.03	—	—	++++	—	++++

THE EFFECT OF NEOSALVARSAN TREATMENT ON THE LATE SEROLOGIC REACTIONS OF PHILIPPINE MONKEYS INOCULATED WITH YAWS OR BOTH YAWS AND SYPHILIS.

By ONOFRE GARCIA

Of the Division of Biology and Serum Laboratory
Bureau of Science, Manila

THREE TEXT FIGURES

The early serologic reaction produced by a primary treponematous lesion is more amenable to treatment than the late one that coincides with the development of full immunity.[1] Monkeys that become serologically negative following treatment may again become serologically positive after reinoculation with either killed or living yaws or syphilis treponema.[2] The duration of the late serologic reaction in Philippine monkeys has been determined in the previous report.[3]

The present experiment has the purpose of studying the effect of the neosalvarsan treatment on some animals that still show strong, positive, late serologic response beyond the limit of time previously tested.

TECHNIC OF STUDY

The history of each monkey is summarized in Table 1. The blood for the serologic reaction was withdrawn through the heart, and the treatment was given intramuscularly. In the beginning of the experiment two treatments were given within two weeks and two successive serologic reactions were performed in the following two weeks. This procedure was, however, altered when the animals showed signs of toxicity and emaciation [4] in which case the bleeding and treatment were made on the same day, and at less-regular intervals as the treatment advanced. The doses, the interval of injections, and the total amount of

[1] Philip. Journ. Sci. 35 (1928) 294.
[2] Philip. Journ. Sci. 35 (1928) 291–311; 42 (1930) 203–211.
[3] Antea.
[4] Philip. Journ. Sci. 35 (1928) 292.

drug received by each monkey are recorded in the accompanying charts (see text figures).

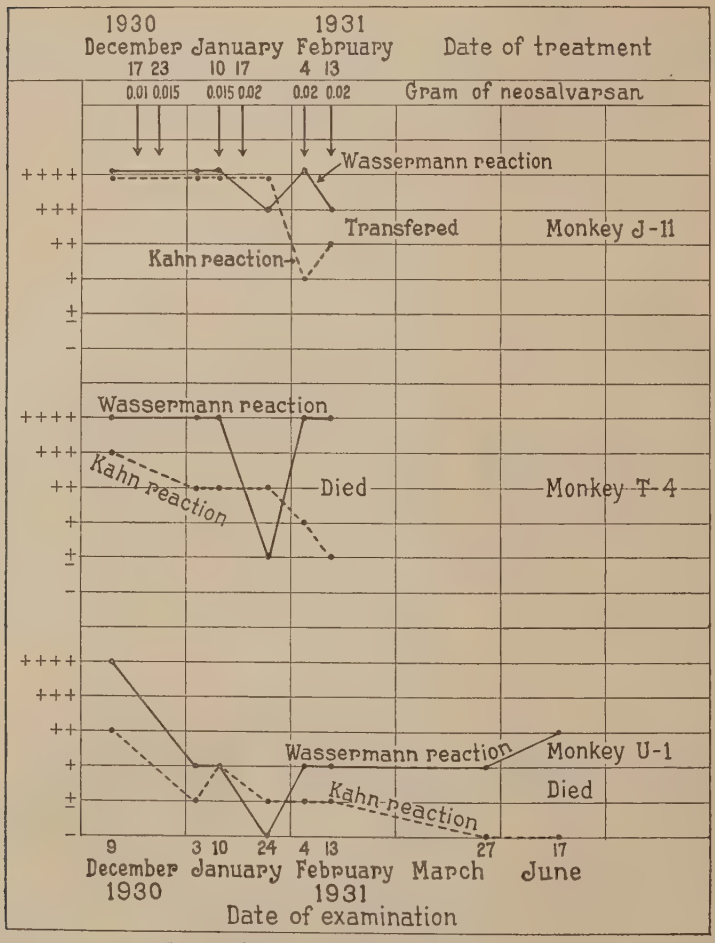

FIG. 1. Charts of monkeys J–11, T–4, and U–1.

The technics for the Wassermann [5] and Kahn [6] tests were the same as in the previous experiments.

[5] Philip. Journ. Sci. § B 12 (1917) 249; 40 (1929) 57.
[6] The Kahn Test, a Practical Guide. Williams and Wilkins Co. (1928).

TABLE 1.—*Showing the number and kinds of injections, period of inoculation or infection, and previous treatment received by the experimental monkeys.*

Monkey.	Duration of infection before treatment.		Number and kinds of inoculations.				Previous treatment with neosalvarsan.		Date of first and last inoculation.	
			Living.		Killed at 60° C. and above.					
			Syphilis.	Yaws.	Syphilis.	Yaws.	Date.	Dose.	First.	Last.
	Yrs.	*mos.*								
W–23	2	5	1	7	0	3	VIII– 3–28 XII–14–28	0.19	I–18–29	II–14–30
W–25	2	5	1	7	0	2	VIII– 3–28 XII–14–28	0.19	I–20–28	II–14–30
J–18	2	4	0	2	0	1			IV–16–28	II–27–29
f–2	2	1	0	2	0	0			VI–25–28	II–27–29
J–11	4	5	2	6	0	0	XII– 2–26	0.09	III– 9–26	I– 7–30
T–4	4	2	2	5	0	0	I–25–27 XII–14–27	0.115	VII– 2–26	I– 7–30
U–1	3	0	1	4	0	3			VII–27–27	II–14–30
j–1	1	9	1	2	0	0			VIII–17–28	VI–22–29
Z–1	1	7	0	1	0	[a]			[b]	VIII– 2–28
K–28	0	7	0	2	0	0	I–21–30 I–28–30	0.03	XI–27–29	III–27–30

[a] Tissue from yaws.

[b] Tissue from yaws; date unknown.

DISCUSSION OF RESULTS

The ten monkeys that were employed in this experiment received more than one inoculation of either yaws or both yaws and syphilis. The duration of the infection was from seven months to over four years. Five of the ten monkeys had neosalvarsan

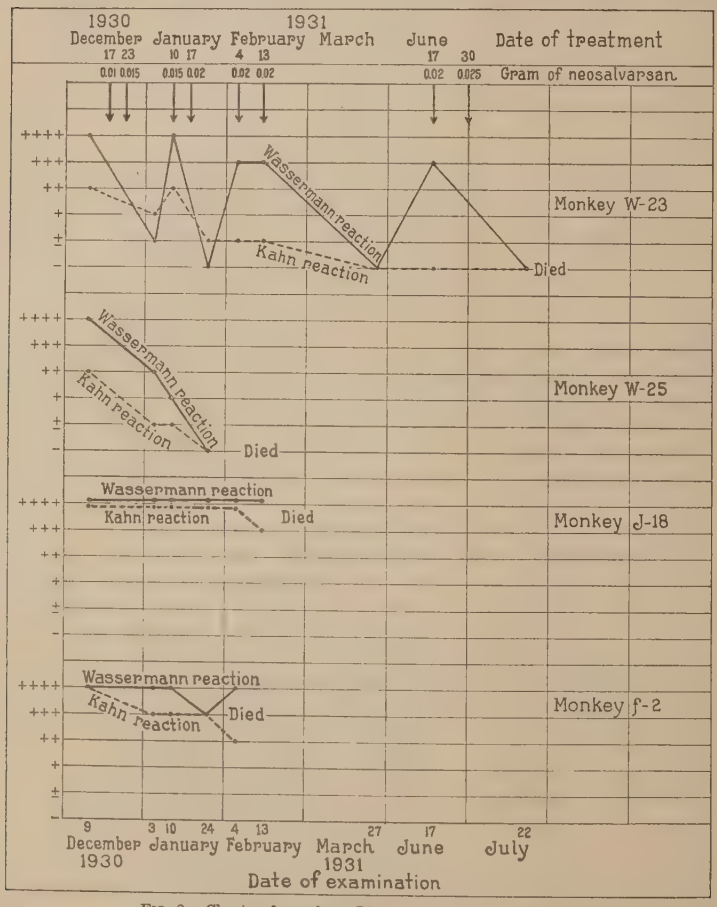

FIG. 2. Charts of monkeys W–23, W–25, J–18, and f–2.

treatment previous to this experiment, and the other five had none. Those that had received previous treatment were, however, reinoculated with yaws and syphilis. Table 2 shows the date, amount of treatment, and the serologic reactions performed before and after the last treatment.

The result of the experiment, as hereby reported, shows that monkeys W–23, W–25, and K–28, which received neosalvarsan previous to the present experiment, responded rather more promptly to treatment than those that did not receive any; monkeys K–28 and Z–1 were the only two animals that survived the nine months' treatment. These two animals presented, however, a different serologic response. Monkey K–28, which received previous treatment, responded to the last treatment sooner than

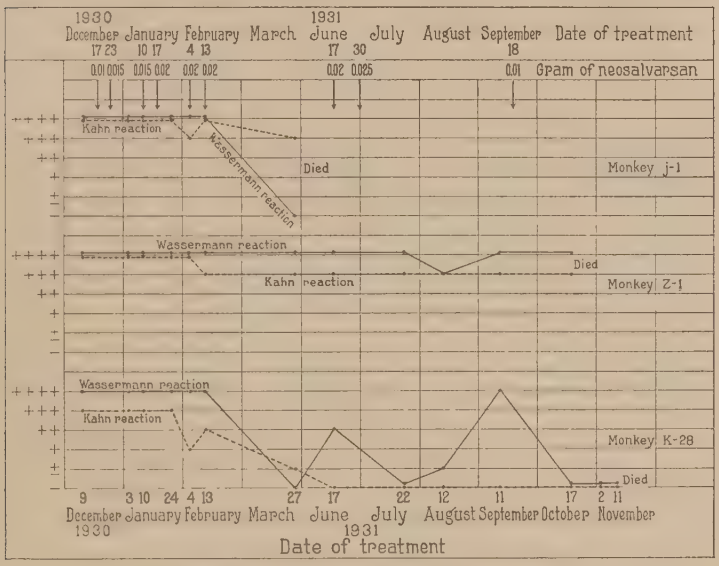

FIG. 3. Charts of monkeys j–1, Z–1, and K–28.

monkey Z–1, which did not receive any. Besides that the period of infection was longer in the latter than in the former (see text figures and Table 2). Monkeys J–18, f–2, u–1, and j–1, which were not previously treated, did not show any remarkable decrease in their serologic reactivity for the period of two to six months of treatment. However, a slight decrease or complete negative serologic reactions were noted in each monkey of the last group in either the Wassermann or the Kahn test. Although monkeys J–11 and T–4 had received previous treatment, the period of infection was extended more in these two animals than in the others and a decrease of serologic reactivity was noted within a period of three months' treatment (see Table 2 and text figures).

TABLE 2.—*Showing the duration of treatment and serologic reaction before and after treatment.*

Monkey.	Duration of infection.	Previous treatment.		Serologic reaction. Before treatment.	
		Year.	Amount.	Wassermann.	Kahn.
	Yrs. mos.				
W–23	2 5	1928	0.19	++++	++
W–25	2 5	1928	0.19	++++	++
J–18	2 4	------	------	++++	++++
f–2	2 1	------	------	++++	++++
J–11	4 5	1926	0.09	++++	++++
T–4	4 2	1927	0.115	++++	+++
U–1	3 0	------	------	++++	++
j–1	1 9	------	------	++++	++++
Z–1	1 7	------	------	++++	++++
K–28	0 7	1930	0.03	++++	+++

Monkey.	After last treatment.		Inoculations received by each monkey.	Duration of treatment.	Remark.
	Wassermann.	Kahn.			
				Months.	
W–23	—	—	11	6	
W–25	—	—	10	2	
J–18	++++	+++	3	2	
f–2	++++	++	2	3	
J–11	+++	++	8	3	Transferred to rat-bite experiment.
T–4	++++	+	7	3	
U–1	++	—	8	3	
j–1	—	+++	3	3	
Z–1	++++	+++	2	9	
K–28	—	—	2	9	

Of the ten monkeys that were studied, only two survived for as long as nine months. Most of the animals died after about the third, fourth, or sixth month, beginning from the first treatment. The high susceptibility of Philippine monkeys to neosalvarsan and the frequent bleedings made on them may have been responsible for their early death. It may be seen in the text figures that the treatment was not regular, due to the fact that the animals that lived more than three months were becoming emaciated and weak.

It became known in previous experiments that the effect of the treatment on the late serologic reaction depends in general on the degree of sensitization resulting from the severity of the

infection, superinfection, or superinoculation and of individual serologic reactivity.[7] These animals that responded to the treatment as shown in the accompanying charts presented irregular curves, which are especially noted with the Wassermann test that was plotted by the reading of the cholesterolized antigen alone. The Kahn test was recorded by the average reading of three tubes.

The plotted serologic curves of each monkey are interesting in that two serologic tests, especially during the period of treatment, may or may not coincide during one simultaneous analysis of the serum (see text figures).

CONCLUSION

The serologic curves of the Philippine monkeys inoculated with yaws or both yaws and syphilis and treated with neosalvarsan at the stage of late serologic reactions can be compared favorably with those observed in man under the same conditions.

Of ten monkeys that were treated and had the infection for about the same length of time, those that had received previous treatment responded more promptly than those that had not received any. In the latter animals, however, there was only a gradual decrease in serologic reactivity with either the Wassermann or Kahn test during a period of two to six months of treatment.

ACKNOWLEDGMENT

I hereby express my sincere appreciation to my chief, Dr. Otto Schöbl, for his kind assistance.

[7] Philip. Journ. Sci. 35 (1928) 261; 40 (1929) 89–90.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Charts of monkeys J–11, T–4, and U–1.
2. Charts of monkeys W–23, W–25, J–18, and f–2.
3. Charts of monkeys j–1, Z–1, and K–28.
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THE EFFECT OF NEOSALVARSAN TREATMENT ON THE LATE SEROLOGIC POSITIVE VERNES, WASSERMANN, AND KAHN REACTIONS IN PHILIPPINE MONKEYS INOCULATED WITH YAWS OR BOTH YAWS AND SYPHILIS.

By Carlos Monserrat

Of the Department of Pathology and Bacteriology, College of Medicine University of the Philippines, Manila

TWO TEXT FIGURES

In a previous communication,(1) the results of the Vernes test performed with blood of Philippine monkeys inoculated with yaws, syphilis, or both, were demonstrated and compared with the results of the Wassermann and Kahn reactions.

The present study has the purpose to show the effect of neosalvarsan treatment on some animals that still show strong positive late serologic response with the three above-mentioned methods.

The changes in the degree of these three reactions, observed during the periods of treatment, were registered and compared by drawing serologic curves.

The majority of the monkeys used in the present experiment were repeatedly inoculated in the past at different intervals with both syphilis and yaws; the rest received inoculations only of living yaws and dead treponemas. One-half of the tested animals received also neosalvarsan injections, but later these monkeys were reinoculated with syphilis or yaws, and at present their blood shows positive reactions with the Vernes, Wassermann, and Kahn tests.

It has been demonstrated that monkeys, which become serologically negative after treatment, may become again positive after reinoculation with either killed or living yaws or syphilis treponemas.(2)

Marked serologic changes in the degree of the late Wassermann reaction have been noticed in human patients with chronic or latent syphilis under the influence of neosalvarsan treatment, especially with the employment of cholesterinized antigens.

These changes were manifested in the form of a strong positive Wassermann, becoming negative after a few injections of neosalvarsan and then becoming again positive with new neosalvarsan injections. This is particularly evident in cases of discontinued neosalvarsan treatment. Furthermore, the "reactivation" of the Wassermann test, as proposed by Millan, with the administration of small doses of neosalvarsan, is a well-known method employed in the serological diagnosis of cases of chronic or latent syphilis.

Syphilitic patients under the influence of neosalvarsan treatment and examined repeatedly with the Kahn test often do not show marked fluctuations in the degree of the serologic reactions compared with the Wassermann test. However, the Kahn reaction remains sometimes persistently positive in well-treated individuals in the absence also of clinical manifestations of syphilis.

Vernes, of the Prophylactic Institute of Paris, developed a new test for the serological diagnosis of syphilis. His method has been tried on a considerable number of syphilitic patients with very good results. The Vernes test is a quantitative flocculation test and has an advantage over the ordinary Wassermann and Kahn methods in that the readings are made more accurate with the photometer and the results are more stable or less fluctuating; therefore, the effect of the different drugs administered during the course of the disease can be easily evaluated by this method. It is important from the therapeutic standpoint to observe in the serologic curve of the patient the effect of each drug administered during the course of a particular treatment. In this respect the Vernes reaction is particularly useful.

MATERIAL INVESTIGATED

Through the courtesy of Dr. Otto Schöbl, formerly of the Bureau of Science, the sera of ten Philippine monkeys, inoculated with syphilis and yaws, were furnished me for the Vernes reaction.

The strain of *Treponema pallidum* employed in the inoculations was the well-known Nichols strain. The strains of *Treponema pertenue* were secured by direct inoculations from patients to the Philippine monkeys. The duration of the infection in the tested animals ranged from seven months to four years five months, and the history of each monkey is summarized in Table 1.

TABLE 1.—*Showing the number and kinds of injections, period of inoculation or infection, and previous treatment received by the experimental monkeys.*

PREVIOUSLY TREATED WITH NEOSALVARSAN.

Designation of monkey.	Inoculations.						Duration of infection up to the time of the present experiment.	
	Living syphilis.	Living yaws.	Yaws killed at 60° C.	First.	Last.	Total.		
							Yrs.	*mos.*
W–23	1	7	3	I–18–29	II–14–30	11	2	5
W–25	1	7	2	I–20–28	II–14–30	10	2	5
J–11	2	6	0	III– 9–26	I– 7–30	8	4	5
T–4	2	5	0	VII– 2–26	I– 7–30	7	4	2
K–28	0	2	0	XI –27–29	III–27–30	2	0	7

NOT PREVIOUSLY TREATED WITH NEOSALVARSAN.

J–18	0	2	1	IV–16–28	II–27–29	3	2	4
f–2	0	2	0	VI–25–28	II–27–29	2	2	1
U–1	1	4	3	VII–27–27	II–14–30	5	3	0
Z–1	0	1	[a]	[b]	VIII– 2–28	2	1	7
j–1	1	2	0	VIII–17–28	VI–22–29	3	1	9

[a] Tissue from yaws.

[b] Tissue from yaws; date unknown.

TABLE 1.—*Showing the number and kinds of injections, period of inoculation or infection, and previous treatment received by the experimental monkeys*—Continued.

PREVIOUSLY TREATED WITH NEOSALVARSAN.

Designation of monkey.	Neosalvarsan treatment previous to the present experiment.			Last serologic reactions before present experiment.			Serologic reactions at the time of the present experiment, December 9, 1930.		
	Last injection.	Total dose.	Duration of treatment.	Date.	Kahn.	Wassermann.	Vernes.	Kahn.	Wassermann.
			Mos.						
W–23	XII–14–28	0.19	6	VI–27–30	+++	++++	5	++	++++
W–25	XII–14–28	0.19	2	VI–27–30	++++	++++	6	++	++++
J–11	XII– 2–26	0.09	3	VIII– 6–30	++	++++	60	++++	++++
T–4	XII–14–27	0.115	3	X–25–30	+	++++	9	+++	++++
K–28	I–28–30	0.03	9	XI–27–29	++	++++	26	+++	++++

NOT PREVIOUSLY TREATED WITH NEOSALVARSAN.

J–18	0	0	0	X–25–30	++	++++	132	++++	++++
f–2	0	0	0	VIII– 6–30	++++	++++	42	++++	++++
U–1	0	0	0	VI–27–30	++++	++++	6	+++	++++
Z–1	0	0	0	V–25–30	+	++++	109	++++	++++
j–1	0	0	0	XII–17–30	++++	++++	57	++++	++++

For the present experiment the animals received several intramuscular injections of neosalvarsan, the injections being administered by Dr. Onofre Garcia, of the Bureau of Science. The doses and intervals of injections and the total amount of drug received by each monkey were recorded in the accompany-

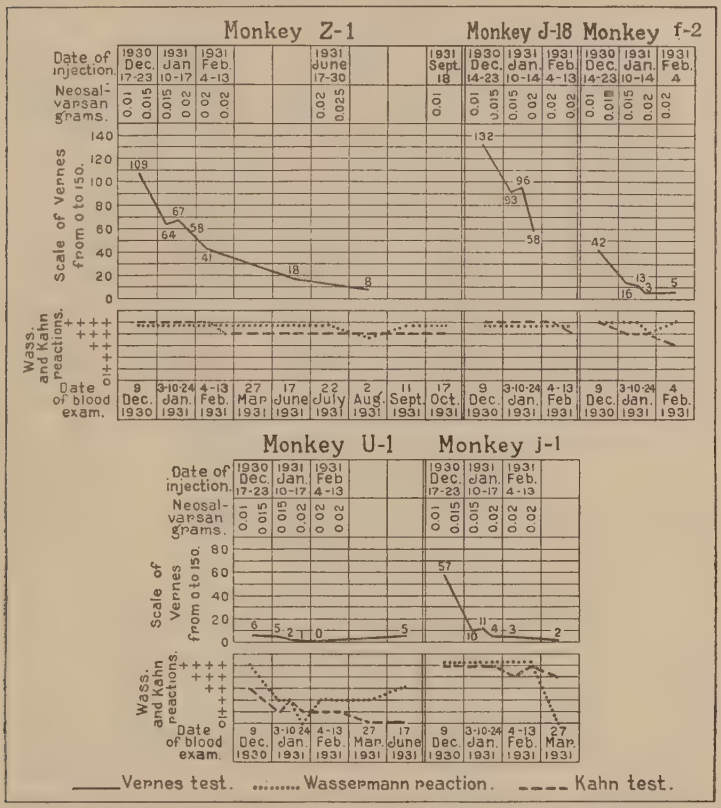

FIG. 1. Chart showing the effect of neosalvarsan treatment on the serologic curves of the Vernes, Wassermann, and Kahn reactions in five untreated monkeys inoculated with yaws and both yaws and syphilis.

ing charts (figs. 1 and 2). At the beginning of the experiment two treatments were given within two weeks and two successive serologic reactions were performed in the following two weeks. Later, due to signs of emaciation and toxicity in the animals, the bleeding and the treatment were made on the same day and with less regular intervals as the treatment advanced.

TECHNIC

The Wassermann and Kahn reactions were performed by Dr. Onofre Garcia, of the Bureau of Science. The Vernes test was performed by the writer.

The technic for the Vernes,(3) Wassermann,(4) and Kahn(5) tests was the same as in the previous experiments.

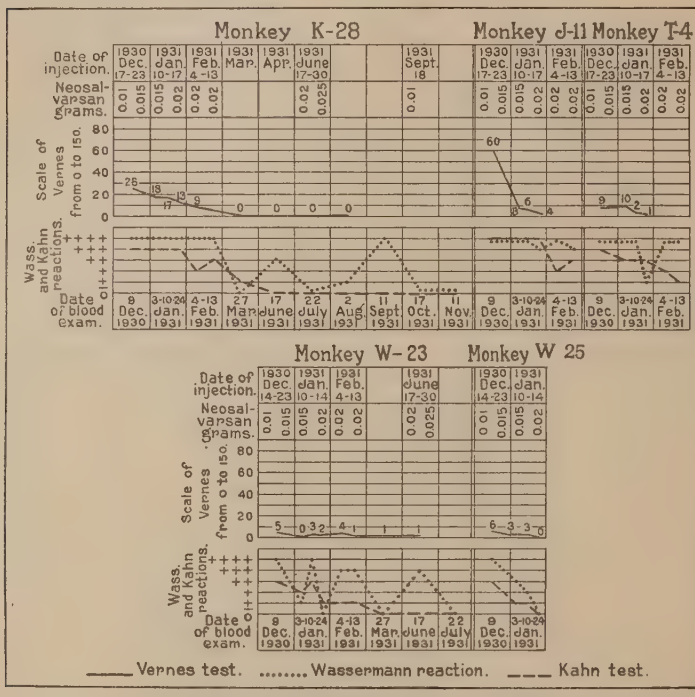

FIG. 2. Showing the effect of neosalvarsan injections on the serologic curves of the Vernes, Wassermann, and Kahn reactions in five monkeys inoculated with yaws and syphilis, which received previously, certain amount of the same drug.

In the Wassermann test the reading was made only from the tube containing the cholesterinized antigen. In the Kahn test the final results were an average of the reading of the three tubes.

DISCUSSION

The results recorded in Table 1 demonstrate that monkeys that did not receive neosalvarsan treatment in the past showed at the time of this investigation stronger positive Vernes, Wassermann,

and Kahn reactions than the previously treated monkeys. In the group of treated animals, it appears that the duration of the infection and the time that elapsed between the last dose of neosalvarsan and the last reinoculation have more influence on the degree of the Vernes and Kahn reactions than the number of reinoculations performed in the same animals.

This is illustrated in monkeys W–23, W–25, J–11, and T–4. Monkeys W–23 and W–25 received more reinoculations (11 and 10), but the duration of the infection was only two years five months. Monkeys J–11 and T–4, on the contrary, received fewer reinoculations (8 and 7), but the duration of the infection was four years five months and four years two months, respectively.

All these monkeys received approximately the same amount of neosalvarsan. Monkeys W–23 and W–25 received the last dose of the drug fourteen months previous to the last reinoculation, while monkeys J–11 and T–4 received their last dose of neosalvarsan two and three years, respectively, before the last reinoculation.

Monkeys W–23 and W–25 at the time of the present experiment showed weaker positive Vernes and Kahn reactions compared with monkeys J–11 and T–4.

The Wassermann reaction in all monkeys treated or untreated was found very strongly positive.

The effect of the present neosalvarsan treatment on the serologic curves of the Vernes, Wassermann, and Kahn reactions in the five untreated monkeys inoculated with yaws and both yaws and syphilis is given in the lower part of fig. 1.

In this figure the effect of each case of neosalvarsan can immediately be appreciated by the descent of the Vernes curve. The Kahn and Wassermann curves on the other hand remained unchanged during a period of three months in three of the infected monkeys (J–18, f–2, j–1) and in one monkey (Z–1) during a period of nine months.

The effect on the serologic Vernes, Wassermann, and Kahn reactions of the present neosalvarsan treatment in five inoculated monkeys, which received previously a certain amount of the same drug, is demonstrated in fig. 2.

This figure shows that the amount of reagins in the blood of each animal indicated by the serologic curve of the Vernes test decreases progressively after administration of each dose of neosalvarsan. On the other hand, the curve of the Wassermann reaction is very irregular; the results being sometimes negative,

sometimes very strongly positive in spite of the continuous administration of neosalvarsan.

This is particularly illustrated in monkeys K–28 and W–23. In these animals the Kahn test behaves almost the same as the Vernes reaction.

SUMMARY

The effects of neosalvarsan injections on the positive serologic Vernes, Wassermann, and Kahn reactions in ten Philippine monkeys inoculated with yaws and both yaws and syphilis are here presented and studied. The duration of the infection in the tested animals was from seven months to four years five months.

Five of these monkeys received, before the present experiment, certain amounts of neosalvarsan. These animals, however, were later reinoculated with syphilis or yaws. Five monkeys received no neosalvarsan injection whatsoever.

All the tested animals showed, at the time of this investigation, positive serologic Vernes, Wassermann, and Kahn reactions. Very strong positive serologic reactions, with all three methods, were particularly observed in the group of monkeys that did not receive previously neosalvarsan injections, with the exception of one monkey (U–1).

The duration of the infection, in the group of treated animals, has apparently more influence upon the degree of the Vernes and Kahn reactions than the number of reinoculations performed in these animals.

Marked serologic changes manifested in various degrees with the Vernes reaction were observed in all monkeys whether treated or untreated during the present course of neosalvarsan treatment. These serologic changes can be detected sooner with the Vernes test than with the Wassermann and Kahn tests. The Vernes reaction became more promptly negative or almost negative in the group of untreated animals.

The serologic curve of the Kahn test resembles the serologic curve of the Wassermann reaction in the group of untreated monkeys. These animals showed, under the influence of neosalvarsan treatment, very little change in the serologic curve of both the Kahn and Wassermann reactions, at least during the first three months following the administration of neosalvarsan.

The serologic curve of the Kahn test resembles the curve of the Vernes reaction only in the group of previously treated animals. It must be mentioned, however, that the Kahn reaction was found by Doctors Schöbl and Garcia to be less sentitive in monkeys than the Wassermann reaction. In human patients,

on the other hand, the Kahn test was found more persistently positive than the Wassermann test.

The serologic curved of the Wassermann reaction, particularly in the group of treated monkeys, was found very irregular and unstable. The Wassermann reaction was found sometimes very strongly positive, sometimes completely negative, during the present neosalvarsan treatment.

CONCLUSIONS

1. The Vernes test was found to be as sensitive a method as the Wassermann and Kahn reactions with the blood of Philippine monkeys inoculated with yaws or both yaws and syphilis.

2. Marked serologic changes, under the influence of neosalvarsan injections, can be more easily demonstrated and evaluated by the Vernes test than by the ordinary Wassermann and Kahn methods.

3. The Vernes reaction is a quantitative reaction and the results are more accurate than those of the ordinary Wassermann and Kahn methods, as the amount of reagin in the blood serum of infected animals can be estimated easily and more accurately by means of a photometer.

Neosalvarsan injections produce, in the blood of infected animals, certain changes in the amount of reagin. In the Vernes reaction these changes are estimated in the photometer and expressed by figures. The combination of figures will form later the serologic curve. The serologic curve in the Vernes reaction is more regular and less fluctuating than the Wassermann and Kahn serologic curves. The changes due to the neosalvarsan injections can be detected sooner and followed more closely in the Vernes reaction than those in the ordinary Wassermann and Kahn methods.

4. The Wassermann reaction apparently is not a good method to study the effect of neosalvarsan injections in the blood of infected monkeys on account of the various fluctuations of the Wassermann serologic curve, particularly in previously treated animals.

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ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Chart showing the effect of neosalvarsan treatment on the serologic curves of the Vernes, Wassermann, and Kahn reactions in five untreated monkeys inoculated with yaws and both yaws and syphilis.

2. Showing the effect of neosalvarsan injections on the serologic curves of the Vernes, Wassermann, and Kahn reactions in five monkeys inoculated with yaws and syphilis, which received previously, certain amount of the same drug.

The data on neosalvarsan treatment and the serological curves of Wassermann and Kahn tests were taken from Doctor Garcia's paper, "The effect of neosalvarsan treatment on the late serologic reactions of the Philippine monkeys inoculated with yaws or both yaws and syphilis," in this issue.

SCLEROTIUM STEM ROT OF DELPHINIUM AND OTHER ORNAMENTAL PLANTS IN TRINIDAD VALLEY, MOUNTAIN PROVINCE, PHILIPPINE ISLANDS.

By T. G. Fajardo

Plant Pathologist, Bureau of Science, Manila

EIGHT PLATES

INTRODUCTION

The annual *Delphinium*, an ornamental plant, like other temperate zone ornamental plants, is grown successfully in Trinidad Valley and other places in the vicinity of Baguio, Mountain Province, Philippine Islands. In this *Delphinium* species and in many other ornamental plants grown in flower beds at the Trinidad Agricultural School, a certain stem and root-rot disease was found in May, 1932. The disease in the annual *Delphinium* species causes yellowing, wilting of the leaves, and finally drying up of the plants due to rotting of the roots and parts of the stem (Plates 1, 2, and 3). On the infected parts of these diseased plants a fungus with coarse white mycelia and numerous small round light brown to dark brown sclerotia, similar to *Sclerotium rolfsii* Sacc., was observed.

In the United States a crown and root rot of *Delphinium* species, *Funkia* spp., *Pentastemon barbatus* Roth, and *Valeriana officinalis* L. was first reported in Connecticut by Clinton(2) in 1907. In 1924, Welch(15) reported a sclerotial disease of cultivated delphinium in New York, Pennsylvania, Indiana, and New Jersey, and proved by inoculation that the organism is pathogenic to both the annual and perennial varieties of larkspur. Because he was unable to identify the fungus with any described species, he proposed the name *Sclerotium delphinii*. Gloyer and Glasgow(3) in 1924 also found the disease to be very abundant on a variety of delphinium from Philadelphia, and ascribed it to *Sclerotium delphinii* Welch. In 1927, Takahashi(12) reported a sclerotium disease of larkspur in Madison, Wisconsin, which in 1925 destroyed 25 per cent of the larkspur in one commercial nursery near Madison. This fungus in culture was similar to or identical with *Sclerotium delphinii* Welch.

Weber(14) in 1931 listed two species of delphinium as host plants to *Sclerotium rolfsii* in Florida. In Trinidad Valley, Mountain Province, Philippine Islands, the annual delphinium was found severely attacked by *Sclerotium rolfsii*, and as far as the writer is aware, the occurrence of *Sclerotium rolfsii* on delphinium in the Philippines has not been reported. It might be interesting, therefore, to report and describe the disease occurring in the annual delphinium and other ornamental plants in Trinidad Valley, the causal organism of which is different from the organism connected with crown and root rot of both the annual and the perennial delphinium in the United States.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

The fungus causing the stem and root-rot of delphinium grown in the Mountain Province, Philippine Islands, is of wide distribution. It has been found attacking various garden and ornamental plants in Trinidad Valley and in the lowland provinces of Luzon. This disease was previously found causing stem and root rot of beans and tomatoes in Trinidad Valley, and in 1932 it was found on delphinium, causing from 5 to 10 per cent infection in some beds, early in the season. Before the end of the season almost 25 to 30 per cent of the plants in the bed were infected and killed. Other ornamental plants, such as petunia, carnation, amarylli lily, and Watsonia, were also affected. In lowland provinces various garden and ornamental plants are found affected. In a flower garden in Manila about 80 per cent of the seedlings of a palm [*Adonidia merrillii* (Becc.) Becc.] were attacked, and a potted Amazon lily (*Eucharis grandiflora* Planch. and Linden) was completely destroyed by this fungus (Plate 8).

GENERAL SYMPTOMS

Delphinium plants infected with the stem and root-rot disease first show yellowing of the lower older leaves. Within a few days the upper leaves progressively turn yellow, with wilting of the terminal shoot, and finally the whole plant dries up and dies (Plates 1 and 2). When the plant is pulled out, a part, or the whole root system, or the stem is found to be either rotted or disintegrated, and on these infected regions there may be white tufts of mycelia or sclerotia (Plate 3). Infection may in certain cases appear on one side of the plant only, but as the fungus advances, the stem, crown, and roots are girdled or de-

cayed, and the infected plant usually topples over and can be pulled up easily, depending upon the stage of the disease. Further evidence of the disease is the appearance of the coarse radiating mycelia or small brown sclerotia of the fungus at or near the infected regions. Under favorable conditions the mycelia run up to the stem, or may be found ramifying on the ground near the infected plants. Numerous small brown sclerotia about the size of mustard seeds may be observed in these regions.

ISOLATION

A pure culture of the causal organism can be readily isolated from the infected plants either by placing in a moist chamber small portions of infected parts and allowing the fungus to develop its aërial fluffy mycelium, or by planting in poured agar plates small bits of infected tissues or mature sclerotia that have been surface-sterilized with 1:1000 mercuric chloride. After two or three days subcultures from the edge of the colony can be made under sterile conditions. The fungus on potato-glucose agar produces a white, fluffy, mycelial growth, and within four to six days light brown to dark brown sclerotia almost the size of mustard seeds are produced (Plate 4).

PATHOGENICITY

The fungus is pathogenic on delphinium and many other plants. Successful infection was obtained when the mycelia or sclerotia were placed near the stem of the experimental plants. Infection was more rapid when the mycelia were inserted into a bruised stem or placed near an uninjured stem than when inoculations were made by placing the sclerotia near the stem. When the air was warm and moist, and the plants inoculated were young and succulent, delphinium plants died within a week, while more-succulent tomato or garden bean (*Phaseolus vulgaris* Linn.) seedlings died within two to five days. In these experiments the fungus was readily reisolated and is the same fungus as originally used.

IDENTITY OF THE FUNGUS WITH SCLEROTIUM ROLFSII

The fungus isolated from delphinium [1] and other flowering plants in Trinidad Valley, Baguio, Mountain Province, behaves in culture like the well-known *Sclerotium rolfsii* Sacc., as described by Higgins,(5) Taubenhaus,(13) Stevens,(11) and others.

[1] An identical fungus has been isolated from bean and tomato plants.

The fungus in culture produces a white and fluffy mycelium, and after four to six days sclerotia appear. The sclerotia produced in culture or those from the field are round, measuring usually from 0.5 to 1.6 mm, with most of the sclerotia falling between 0.8 and 1 mm. The sclerotia are at first white, become cream-colored, and finally turn light brown to dark brown. This fungus, compared in culture with the three authentic strains of *Sclerotium delphinii* from the United States, differed only in size and in the number of sclerotia. Gloyer and Glasgow(3) give dimensions of *S. delphinium* from pinhead size to as large as from 5 to 10 mm in length and about 2 to 3 mm in thickness. The sclerotia of the three *S. delphinii* strains cultured on potato-glucose agar are large and pitted, and vary from 1.2 to 4.5 mm long by 1.2 to 2.5 mm wide for Illinois strain I, 2.1 to 6.5 mm long by 1.6 to 3.5 mm wide for Illinois strain II, 2.5 to 5.0 mm long by 2.0 to 3.9 mm wide for Illinois strain III, and 1.8 to 8.5 mm long by 1.5 to 4.5 mm wide for Wisconsin 517 strain (Plates 5 and 6). The number of sclerotia produced by *Sclerotium rolfsii* varies from 100 to 400 or more, depending upon the media used, and in *S. delphinii* the number varies from 10 to 50, but in no case was it found to produce over 100 sclerotia in a single colony (Plate 7). From these findings it is evident that the organism causing crown and stem rot of delphinium in the Mountain Province, Philippine Islands, is *Sclerotium rolfsii* Sacc., and is, therefore, different from *S. delphinii* Welch, the organism causing crown and stem rot of delphinium in the United States. Furthermore, this fungus was compared with authentic cultures of *S. rolfsii* from the United States and was found to be identical with the latter.

CERTAIN FACTORS INFLUENCING THE DISEASE

The disease is more prevalent in light sandy soil than in heavy soil, and is favored by warm, moist weather. When the disease is established it spreads along the row in both directions, from one plant to another by migration of the fungus mycelium, or by transfer of the mycelia or sclerotia from one part of the field to another through cultivation. Air and moisture are necessary for infection. If the organism is buried deeply in the soil it becomes less active. The organisms can live saprophytically in the soil in its mycelial stage, and can resist unfavorable weather with its sclerotial bodies.

HOST RANGE OF SCLEROTIUM ROLFSII

This fungus, being one of the soil fungi attacking a wide variety of host plants, was reported serious on tomato by Halsted(4) in 1892 and by Rolfs(10) in 1893. Since that time various writers have reported it on various plants so that now it is known to be widespread in tropical and subtropical countries. Weber(14) in 1931 listed 189 host plants attacked by this fungus; *Delphinium ajacis* Linn. and *D. grandiflorum* Linn. are among the ornamental plants listed as infected in Florida. In the Philippines, Reinking(8, 9) reported a *Sclerotium* causing damping off of seedlings and stem rots in peanuts, coffee, rice, tobacco, garden bean, gabi [*Colocasia esculentum* (L.) Schott], soy bean, sweet potato, and yautia (*Xanthosoma sagitifolium* Schott). *Sclerotium rolfsii* was reported by Pereira(7) on Philippine rice, and in 1924 Ocfemia(6) found it causing fruit rot of tomato and pepper. In 1927, Atienza(1) found it on sitao (*Vigna sesquipedalis* Linn.), patola [*Luffa cylindrica* (L.) Roem.], Jerusalem artichokes (*Helianthus tuberosus* Linn.), "pongapong" [*Amorphophallus campanulatus* (Roxb.) Blume], *Caladium* spp., sugar cane, avocado (*Persea americana* Linn.), and squash (*Cucurbita maxima* Duch.). In a field survey made by the writer in 1930 and on various field trips made thereafter, the above-mentioned plants were found infected by *Sclerotium rolfsii*, and in addition, *Delphinium* species, carnation, amarylli lily, Watsonia, petunia, Amazon lily (*Eucharis grandiflora* Planch. and Linden) Plate 8, and "bunga de China" [*Adonidia merrillii* (Becc.) Becc.] were also affected by the same fungus. As the climate is favorable to fungus activity in the Philippine Islands, other ornamental and garden plants not heretofore reported might also be attacked by *S. rolfsii* in other localities.

SUGGESTION FOR CONTROL

The fungus *Sclerotium rolfsii* is readily transported from one locality to another in its mycelial or sclerotial stage, either with the soil, with the seedlings, or with seed boxes. Its spread in the field is accomplished by cultivation and excessive use of water. As this disease is rather difficult to control once it gets started, care must be taken to avoid its introduction to new localities. Infected plants and the soil around them should be removed and burned. Since the parasite becomes less active when it is buried deep in the soil, plowing deep or turning over

the surface soil to a depth of about 8 to 12 inches will greatly minimize the prevalence of the disease. For small infected flower beds or infected soil in pots, sterilization of the soil by firing or changing it with fresh uninfected soil is highly recommended. No chemical has yet been found to be effective in checking the advances of the pathogene once a plant has become infected.

SUMMARY

1. The crown and root-rot disease of delphinium due to *Sclerotium rolfsii* is reported for the first time on *Delphinium* sp. in Trinidad Valley, Mountain Province, Philippine Islands, causing a 5 to 10 per cent infection of delphinium plants in the flower beds at the Trinidad Agricultural School. Other ornamental plants were also infected, but the disease was more serious on the annual delphinium.

2. Infected plants first show yellowing of the lower leaves, but as the disease progresses, the upper leaves also turn yellow, the terminal shoots finally wilt, and within a few days the plants topple over, dry up, and die. The crown and roots of such plants either disintegrate or decay; the plants can be pulled up readily.

3. A pure culture of *S. rolfsii* was isolated and its pathogenicity was proven on delphinium and other garden plants, causing death within a week after inoculation.

4. The fungus in culture is identical with the authentic culture of *Sclerotium rolfsii* Sacc., from the United States, but differs from *S. delphinii* in the size, number, and markings of the sclerotia.

5. The disease is favored by warm and moist weather, and is more serious and prevalent in light, sandy soil than in heavy soil.

6. The fungus is known to attack 189 host plants. In the Philippines, in addition to various garden and field crops, it attacks *Delphinium* sp., carnation, amarylli lily, Watsonia, and petunia at Trinidad Agricultural School; and Amazon lily and "bunga de China" [*Adonidia merrillii* (Becc.) Becc.] in flower gardens in Manila.

7. No control measure has been worked out, but recommendations are made to prevent and minimize the occurrence of the disease.

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ILLUSTRATIONS

PLATE 1

Delphinium in a flower bed infected with *Sclerotium rolfsii* Sacc. Note the yellowing, wilting, and dying condition of the plants. The fungus mycelia and sclerotia are very evident on the infected stem.

PLATE 2

Delphinium plants infected with *Sclerotium rolfsii.* The plants in the foreground are dead or dying, while those in the background are still healthy. Later these plants also were infected and died.

PLATE 3

Delphinium plants infected and killed by *Sclerotium rolfsii* Sacc. Note the complete rotting of the tissues of the crown and roots. The white tufts of mycelia and the young sclerotia of the causal fungus are quite evident on the infected regions.

PLATE 4

Pure culture of *Sclerotium rolfsii* isolated from infected delphinium plants. Note the characteristic light to dark brown sclerotia about the size of mustard seeds.

PLATE 5

Sclerotium rolfsii isolated from delphinium compared with three strains of *Sclerotium delphinii* from the United States. Fig. 1, *Sclerotium rolfsii.* Figs. 2, 3, 4, strains of *Sclerotium delphinii* from Illinois. Fig. 5, *Sclerotium delphinii* from Wisconsin. Note the difference in the number and size of sclerotia between *S. rolfsii* and *S. delphinii; S. rolfsii* sclerotia are smaller and numerous, those of *S. delphinii* are larger and fewer, and are "pitted."

PLATE 6

Photograph showing the relative sizes of sclerotia produced by *Sclerotium rolfsii* and *Sclerotium delphinii.* Fig. 1, *Sclerotium rolfsii.* Fig. 2, *Sclerotium delphinii* Wisc., strain 517. Figs. 3, 4, and 5, *Sclerotium delphinii,* Illinois strains I, II, and III.

PLATE 7

Sclerotium rolfsii and *Sclerotium delphinii*, "Wisconsin 517" on potato glucose agar showing the marked differences between the two organisms (7-day-old colony).

PLATE 8

A potted Amazon lily (*Eucharis grandiflora* Planch. and Linden) infected with *Sclerotium rolfsii*. Note the course white mycelia and the sclerotia of the fungus. This plant was from a flower garden in Manila.

PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

PLATE 5.

PLATE 6.

PLATE 7.

PLATE 8.

THE ROOT-KNOT NEMATODE, HETERODERA RADICICOLA (GREEF) MULLER, OF TOMATO AND OTHER PLANTS IN THE PHILIPPINE ISLANDS.

By T. G. FAJARDO[1]

Plant Pathologist, Bureau of Science, Manila

and

M. A. PALO

Junior Mycologist, Bureau of Science, Manila

EIGHT PLATES

INTRODUCTION

The disease, generally known as root knot, caused by *Heterodera radicicola* (Greef) Muller,[2] has been known to be a serious pest of crop plants. In some countries the pest is so prevalent that susceptible crops have to be abandoned and thousands of dollars spent for steam and chemicals in attempts to control the disease. In the United States, especially in the sandy truck and cotton regions of the Southern States, the root knot is a serious outdoors disease. Thus, in Florida, Godfrey (9) reported that in one county the loss on snap beans, cabbage, celery, eggplant, potatoes, lettuce, peas, and tomatoes amounted to 150,000 dollars. Gilbert (8) also reported that the loss due to the nematode for the entire cotton belt in 1917 was estimated at approximately

[1] Read before the First Philippine Science Convention, Manila, March 16, 1932. An abstract was published in the proceedings of the society with the permission of the Director of the Bureau of Science.

[2] Cobb (5) in 1924 proposed the name *Caconema radicicola* (Greef) Muller for this species. However, Goodey (12), discussing the nomenclature of the root-knot nematode, states that, according to the law of priority of the international rules of zoölogical nomenclature, the nematode named by Greef as *Anguillula radicicola* (= *H. radicicola* of Muller) should have the generic name *Anguillulina* and, therefore, should be called *Anguillulina radicicola* Greef. Because of the confusion that might arise in literature and for the purpose of this article the well-known binomial of the pest, *Heterodera radicicola* (Greef) Muller, is retained.

200,000 bales of cotton and 100,000 tons of seed worth 16,000,000 dollars.

In the Philippines the root-knot nematode is common and widespread, attacking abacá, sugar cane, tobacco, tomato, beans, cowpeas, "sitao," garden beets, celery, pepper, sweet potato, and some weed plants. Because the climate of the Philippines is favorable to the continued activity of the parasite, and since all important truck and field crops are now affected, it is feared that the disease may become more widespread and serious and limit the cultivation of the important garden and field-crop plants, as it has in other countries. The results of our studies of the disease, especially as it affects the tomato, one of the major commercial truck and field crops in the Philippines, are here presented.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

Berkeley(1) first reported the disease in Europe in 1855. Since then various workers have studied and reported the disease as attacking various agricultural plants, and now it is known to have a world-wide distribution and to attack over five hundred species of plants. In the Philippines, the disease was reported by Reinking(17) on tobacco and sugar cane in 1916, by David and Roldan(6) on tobacco in 1926, and by Ocfemia and Calinisan(16) on abacá in 1928. Dr. M. M. Alicante, formerly soil chemist of the Bureau of Science, informed the writers that during his soil survey in Cagayan Valley in 1926, he found the root-knot nematode common and its infestation serious on some old tobacco farms along the river banks.

In a plant-disease survey made by the senior writer during the summer of 1930, and in various field trips in 1931 and 1932 through the northern and central provinces of Luzon, the root-knot nematode was found more serious in light sandy soil than in any other kind of soil, affecting various crop and weed plants. In Binalonan, Pangasinan Province, 100 per cent of the garden beets grown in the old school garden were infected and badly attacked, so that the roots were badly deformed and unmarketable. At the Central Luzon Agricultural School, Muñoz, Nueva Ecija Province, a plot of cowpeas and "sitao" showed 100 per cent infection and the plants yielded only a few pods. The disease was also found on the "American" celery in the Chinese gardens in Manila, in the seedbeds in Trinidad Valley and Camp 7, Baguio, Mountain Province, and on tomato, sweet potato, and *Phaseolus lathyroides*, from our new pathological plots in Ma-

nila and in Alabang, Rizal Province. On tobacco and tomato the disease was found in Ilocos Norte, La Union, Pangasinan, Bulacan, Nueva Ecija, Rizal, Laguna, and Batangas Provinces, causing considerable damage. In Cagayan Valley, especially in the light sandy soils, the disease is so important on tobacco that from 50 to 100 per cent infection is not uncommon in most tobacco fields. No data on the aggregate loss due to the disease can be presented, but where it is bad and widespread it is a limiting factor in crop production. The extent of distribution is not completely known, but as the climate of the Philippines is ideal for the pest, it might be found in other islands and provinces not heretofore reported.

SYMPTOMS

The symptoms of root knot on tomato, tobacco, cowpea, or other plants suffering from the disease are quite identical. In general, the infected plants are dwarfed or stunted and wilt or "flag" during the hot dry part of the day, but recover towards evening when the temperature becomes lower. The most characteristic symptom of the disease is the production of galls or abnormal swellings on the root system (Plates 1, 2, 3, and 4). These galls may assume various shapes and sizes, or may coalesce together giving the root system a knotty, swollen, or thickened appearance. The presence of these galls on the roots interferes with the normal physiological functions of the roots, which results in wilting even when the soil is moist. Because of this pathological condition of the root the plant in time ceases to grow and may die prematurely. Plants at any stage of growth are susceptible to infection. Young plants infected early and severely show yellowing of the first few leaves, cease growing, and finally die before they develop many leaves (Plate 6, fig. 3, and Plate 8). Older plants infected during their premature or mature stage may continue to grow, with little or no effect on the growth, and the production of fruit may be reduced but slightly. These plants may be recognized in the field by their pale to yellowish green leaves; upon pulling, galls are found in the root system. If some of these galled roots are split open, the eggs or larvæ of the causal nematode are found.

CAUSAL ORGANISM

The root-knot nematode found on tomato, tobacco, and other plants in the Philippines is the well-known *Heterodera radicicola* (Greef) Muller.

Eggs.—The eggs are almost elliptical or ovoid. Bessey(2) gave measurements for the eggs as 85 to 95 μ long by 34 to 40 μ wide, with an absolute average of 92 by 38.4 μ. Byars(3) states that the eggs are 88 by 34 μ, while Godfrey(9) found the eggs to be about 100 μ. Ocfemia and Calinisan(16) found that the egg of the nematode from abacá is from 76 to 108 μ long by 36 to 48 μ wide, and the average 91.8 by 41.3 μ. Measurements of one hundred eggs from the galls of the infected roots of tomato showed that they vary from 81.7 to 95.0 μ in length and 34.2 to 43.7 μ in diameter, with an average of 90.3 μ long and 39.4 μ wide.

The larvæ.—The larvæ are small slender worms, blunt at the anterior and tapering at the posterior end to a pointed tail. Bessey(2) found them to be 375 to 500 μ in length and about 12 to 15 μ in greatest thickness. Byars(3) found them to be 400 μ in length, while Godfrey(9) found them to be $\frac{1}{80}$ to $\frac{1}{50}$ of an inch, or about 312.5 to 500 μ long, and the diameter about $\frac{1}{30}$ of its length, or about 10.4 to 16.6 μ. Ocfemia and Calinisan(16) found that the nematode larva from abacá measures 331.6 to 464.2 by 16.2 to 25.9 μ, and the average size is 409.5 by 18.8 μ. Our measurements of eighty young larvæ from galled tomato roots showed that they are from 310 to 465 μ long and 12.5 to 17.1 μ thick, or an average of 387.5 μ long and 15.2 μ thick.

Mature female.—The mature female is usually flask- or pear-shaped, and glistening pearly white; it can be seen embedded in the galled tissues of the root. Bessey(2) states that it is 400 to 1,300 μ in length and 270 to 500 or even 700 μ in the greatest diameter, and the average measurements about 800 μ in length and 500 μ in diameter. Byars's(3) measurement for the mature female is about 0.5 millimeter, or 500 μ, while Godfrey(9) states that it is from $\frac{1}{40}$ to $\frac{1}{25}$ of an inch, or about 625 to 1,000 μ. Ocfemia and Calinisan(16) found the mature female nematode on abacá to be from 720 to 1,280 μ in length and 400 to 800 μ in diameter. The writers found that in one hundred mature females the range was from 775 to 1,271 μ in length and 418 to 667.07 μ at the greatest diameter, or an average of 917 by 527 μ.

Mature male.—The mature males are long, slender, and worm-like and can be distinguished from the larvæ by their size and striated cuticle. They are not easy to find, and out of several attempts we were able to measure only three males. They were from 992 to 1,550 μ long and 38.7 to 40 μ thick. Bessey(2)

states that they are usually from 1,200 to 1,500 μ long and 30 to 36 μ thick. Byars(3) reports that the mature male is a little more than 1 millimeter long, while Godfrey(9) found it to be from $\frac{1}{20}$ to $\frac{1}{16}$ of an inch long, or about 1,250 to 1,562.5 μ, and not over one-fortieth as thick as long, or about 31.25 to 39 μ in thickness.

From the above measurements it is evident that the size of the eggs, larvæ, mature female, and mature male of the nematode causing root knot on tomato plants, as here reported, lies near or within the measurements for *H. radicicola* studied by Bessey,(2) Byars,(3) Godfrey,(9) and Ocfemia and Calinisan.(16) Therefore, it is not unsafe to assume that the nematode in question is the well-known root-knot nematode *H. radicicola* (Greef) Muller. In these studies no attempt has been made to examine the internal structure of the pest, but specimens were shown to Dr. M. Tubangui, parasitologist of the Bureau of Science, who confirmed our determination. Plate 5 shows the stages in the life cycle of the root-knot nematode.

PATHOGENICITY TEST

Tomatoes or other susceptible plants when grown in nematode-infected soil are readily infected and show characteristic nematode galls on the roots. In a set of experiments, larvæ in water suspension were poured near the stems of tomato, tobacco, and cowpea plants growing in 8-inch pots. When after twenty-five days these plants were lifted, galls were already produced on the roots, while the check plants, in soil to which the nematode was not added, showed no galls on their root systems. Microscopic examination of the galled roots showed the presence of the root-knot nematode, *H. radicicola.* In another trial tomato seeds were planted in soil mixed with galled roots, and after thirty-five days when the seedlings were pulled up abundant galls were found on the root system.

ARE THERE HETERODERA RADICICOLA STRAINS?

In the Philippines the nematodes causing root knot on tomato, tobacco, table beet, cowpea, celery, and a legume weed, *Phaseolus lathyroides* Linn., are morphologically alike and perhaps of the same physiological strain. In cross-inoculation experiments the nematode from tomato, tobacco, and cowpea can easily infect tomato, tobacco, cowpea, beet, celery, and *Phaseolus lathyroides,* while the nematode on garden beet, celery, and

P. lathyroides can likewise readily infect tomato, tobacco, cowpea, beet, celery, and *P. lathyroides*. The galls produced as a result of infection varied only slightly in size and form. In these experiments galled roots were mixed thoroughly with the sterile soil in which seedlings or seeds were planted.

Further experiments were conducted in which samples of nematode-infected soils from Los Baños, Laguna Province, Binalonan, Pangasinan Province, and from the pathological plot of the Bureau of Science, Manila, were planted with five tomato varieties. When the plants were examined thirty-five days after planting all the plants gave 100 per cent infection. The galls produced in the roots were identical. The results of the above experiments indicate that there is probably but one common species of *H. radicicola* attacking these crops in the Philippines. This conclusion is in agreement with that of Bessey,(2) who found that the root-knot nematodes were mutually interchangeable on the plants he experimented with, and that the root-knot nematode is not markedly differentiated into strains peculiar to certain hosts.

TABLE 1.—*Cross-inoculation experiments using nematode-infected soil from three localities.*

Tomato varieties tested.	Sources of nematode-infeted soil.					
	Pathological plot, Bureau of Science.		Los Baños, Laguna Province.		Binalonan, Pangasinan Province.	
	Plants inoculated.	Plants infected.	Plants inoculated.	Plants infected.	Plants inoculated.	Plants infected.
American varieties:						
Perfection	5	5	11	11	5	5
Matchless	5	5	4	4	5	5
Native varieties:						
Balayan	5	5	5	5	5	5
Laguna	5	5	5	5	5	5
Wild cherry (Lemery)	5	5	11	11	5	5

INFECTION EXPERIMENTS

Amount of moisture and nematode infection.—Godfrey(10) found that moisture plays a small part in the development of root knot so long as the moisture content of the soil is favorable to the growth of the crops. He obtained root-knot infection in infested soil with a water-holding capacity ranging from 20 to 100 per cent, but serious infection was observed

where the soil moisture was best for plant growth. Within the range of 40 to 80 per cent of the water-holding capacity of the soil root knot was abundant, but there was very little difference in the root-knot development. Below 40 per cent, which is too dry for good growth of ordinary plants, and above 80 per cent, which is more or less muddy, considerable root knot is still found. Jones(13) likewise found that infection with nematode was obtained in soil with a moisture ranging from 10 to 100 per cent.

The results of experiments conducted in the greenhouse also showed that root-knot infection can be obtained even when the soil is saturated with water or kept quite dry but still wet enough to maintain plant growth. Eight pots containing infested soil were placed in a shallow galvanized-iron can filled with water. The soil was therefore very wet. After one, two, three, four, and five days tomato seedlings were planted. At the end of thirty-five days from the date of planting all showed nematode-gall infection.

In the other experiment, pots with infected soil were planted with tomato seedlings, and allowed to grow in the greenhouse with only a small amount of water added once every few days to keep the plants from dying. Again, when the plants were examined forty days later, all gave 100 per cent infection.

The effect of flooding on nematode infection.—Jones(13) found that twenty-eight days of continuous flooding were not sufficient to destroy the nematode in gall-infested soils. In a series of experiments nematode infection was obtained even after infected soil had been flooded for thirty-five days. In these experiments, uniformly infected soil was put in 1-cubic-foot cement crocks, flooded with sterile water until the soil was submerged 2 inches, and the water allowed to stand for 1, 5, 10, 20, 25, 30, and 35 days in the greenhouse. At the end of each flooding interval, the water was drawn off by a drainage hole at the bottom. After twenty-four hours of draining, with the soil still wet, a layer of sterile soil was added on the surface where the tomato seeds were planted. After planting, and even during the course of observation, no water was added as the soil was still moist. As checks of the above experiments, crocks of the same size and capacity were filled with the same infected soil sample, kept moist, and planted with tomato seeds at the beginning and at the end of the experiment. Thirty-five days from the date of planting the plants were lifted carefully and the roots examined.

As shown in Table 2, nematode infection was obtained even after infested soil had been flooded for thirty-five days. The percentage of infection, however, decreased to 39 per cent with an average of 0.6 gall per plant, while the checks which were planted at the beginning and at the end of the experiment showed 100 and 96 per cent infection with 7.9 and 3.2 galls per plant, respectively. Flooding at shorter intervals showed abundant gall formation, but a gradual decrease in the percentage of infection was noted.

In another set of experiments, infected soil was put in a large galvanized can and then submerged in water for 25, 30, 35, and 40 days. After each time interval, a portion of the flooded soil was put in 8-inch pots. These pots were allowed to drain for forty-eight hours before tomato seeds were planted. At the end of thirty-five days the plants were examined, and again a high rate of infection was obtained after flooding the soil for twenty-five days, but at the end of thirty-five and forty days the infection was greatly reduced.

The results of the above experiment show that a majority of the nematodes on badly infested soil can be killed by flooding, and that after thirty-five and forty days of flooding infection is greatly reduced. No attempt has been made to determine how long infected soil can be freed by flooding, but it seems that longer flooding periods would completely eliminate nematodes in the soil. Observation in the Philippines seems to substantiate this opinion, as in the case of rice fields kept under water for two to four months, which are usually free from the root-knot nematode.

TABLE 2.—*Effect of flooding infected soil on the root-knot nematode.*

Treatment of soil.	Plants examined.	Infection.	Average number of galls.
		Per cent.	
Not flooded [a]	102	100.0	7.9
Flooded 1 day	130	85.4	4.1
Flooded 5 days	48	83.3	2.6
Flooded 10 days	66	86.3	4.2
Flooded 15 days	47	83.0	2.7
Flooded 20 days	106	75.5	1.4
Flooded 25 days	68	64.0	1.3
Flooded 30 days	62	77.0	3.8
Flooded 35 days	66	39.0	0.6
Not flooded [a]	76	96.0	3.2

[a] Check. The soil for check crocks was taken from the same soil sample and was kept moist, but not flooded.

The effect of desiccation on root-knot infection.—The results of a series of experiments show that nematodes can be killed by desiccation in the soil. The length of time required to kill all the nematodes, however, seems to depend upon the moisture content of the soil. Jones(13) found that galls put in air-dry soil for one and one-half to two weeks failed to produce infection when susceptible plants were planted.

In two series of experiments uniformly infected sandy-loam soil of known percentage of moisture, was mixed thoroughly with infected roots, put in 8-inch pots, and allowed to dry in the greenhouse for various lengths of time up to thirty-five days. At the end of each drying period, the percentage of moisture still remaining in the soil was determined, and then tomato seeds were planted. After the seeds had been planted, the soil was always kept moist to keep the plants in good growth. The plants were examined after thirty-five days from the date of planting. Table 3 is a summary of the results obtained.

TABLE 3.—*Effect of desiccating nematode-infected soil in the greenhouse and root-knot infection on tomato seedlings.*

Series and sample.	Duration of air drying.	Moisture in the soil sample.[a]	Plants examined.	Root-knot infection.
	Days.			*Per cent.*
Series I:				
0	Check.	[b] 11.0		
1	2	6.6	25	100
2	5	4.5	30	100
3	10	2.8	25	100
4	15	2.2	20	100
5	20	2.1	30	100
6	25	2.0	30	16.7
7	30	2.0	25	0
8	35	2.0	25	0
Series II:				
0	Check.	[b] 15.1	75	100
1	10	8.1	75	100
2	15	6.9	75	100
3	20	6.1	50	100
4	25	5.6	75	100
5	30	4.9	70	100
6	35	2.6	135	2.2

[a] The per cent of moisture present in the sample of soil after drying in the greenhouse for a given number of days.

[b] Moisture present in the soil sample before drying in the greenhouse.

In series 1, only 16.7 per cent infection was obtained at the end of twenty-five days, and no infection was found after thirty or thirty-five days of drying. In series II, it will be noted that

100 per cent infection was obtained in soils after drying for thirty days, but after thirty-five days the infection decreased to 2.2 per cent. The difference in the length of time required to free the soil from nematodes in this case is apparently due to the difference in the percentage of moisture in the soil when the experiments were started. While our results differed from those of Jones,(13) it is believed that this discrepancy is due to the initial soil moisture when the experiments were started. In our experiments nematodes were either eliminated, or a large percentage of them were killed within twenty-five and thirty-five days of drying. In one case no infection was obtained after drying potted infected soil for thirty days in the greenhouse.

BIOLOGY OF THE LARVÆ

Sensitiveness of larvæ to complete dryness.—Larvæ are very sensitive and are readily killed by desiccation. In a series of experiments one drop of larvæ in water suspension was placed on glass slides and allowed to dry in the laboratory at room temperature for 1, 3, 4, 5, 6, 7, 8, 10, and 15 minutes. In these experiments, time was recorded when the slide was free from visible water and then further examined under a microscope for free water. After each time exposure, the slides were moistened with a drop of distilled water and kept moist in a Petri dish for forty-eight hours, after which they were examined for dead larvæ. As shown in Table 4, the larvæ were not killed after they had been exposed to dryness for three and four minutes at room temperature. After 5, 6, 7, and 8 minutes of drying, however, 10.1, 47.0, 70.0, and 90.7 per cent, respectively, were found dead. After 10, 15, and 20 minutes, however, all the larvæ were dead and remained dead even when allowed seventy-two hours in moist condition to recover. These dead larvæ showed no motion and were either straight or slightly curved. The check slides, in which the larvæ were kept moist all the time in a Petri dish, were all alive at the end of ninety-six hours, after which they were discarded. In another series of experiments, young larvæ just out of the egg sacs were exposed to the same treatment. Desiccation at room temperature for two minutes did not kill them all, but they were killed in three minutes, confirming the results of Godfrey and Hoshino.(11)

The results, therefore, show that the time required to kill the larvæ depends upon the stage of maturity, the very young larvæ being more sensitive than older ones.

TABLE 4.—*Sensitiveness of root-knot nematode larvæ to desiccation at room temperature (28–32° C.).*

Exposure.	Larvæ under observation.	Recovery of larvæ after they were placed in moist condition for 48 hours.	Larvæ killed in the treatment.
Mins.		*Per cent.*	*Per cent.*
1	8	100.0	0.0
3	5	100.0	0.0
4	5	100.0	0.0
5	18	89.9	10.1
6	17	53.0	47.0
7	13	30.0	70.0
8	15	9.3	90.7
9	20	0	100.0
10	25	0	100.0
15	25	0	100.0
20	20	0	100.0

Longevity of larvæ in water.—Larvæ are more tolerant to moist conditions without food than to desiccation. Larvæ in water suspension were placed on glass slides and kept moist in the laboratory at room temperature, and only a few survived at the end of thirty days in this condition without food. At the end of 18, 24, 30, and 35 days, 73.9, 92.2, and 100 per cent dead larvæ were found from one slide, while on another slide 42.6, 84.4, and 100 per cent larvæ were dead at the end of 18, 24, and 30 days.

In another series of experiments, a drop of larvæ in water suspension was placed in each of four concave glass slides and kept in the laboratory for 3, 8, 14, 25, and 30 days in a moist condition. In order to keep the slides moist, they were put in moist Petri dishes and a drop of water was added into each dish from time to time. At the end of twenty-five days the first three slides had 100 per cent dead larvæ, while slide IV had 90.1 per cent. At the end of thirty days this slide showed 100 per cent dead larvæ. The results show that the larvæ, which

are sensitive to drying, can live for twenty-five to thirty days in a moist condition without food in the laboratory (Table 5). It was observed that larvæ of the younger stage or larvæ that are newly hatched died very much sooner than larvæ of the older stages.

TABLE 5.—*Longevity of larvæ in water.*

Slide No.	Larvæ used.	Dead larvæ after—				
		3 days.	8 days.	14 days.	25 days.	30 days.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
I	26	46.2	61.5	92.3	100	100
II	20	55.0	75.0	85.0	100	100
III	40	57.5	67.5	85.0	100	100
IV	70	24.3	32.9	62.8	90.1	100

Overwintering the root-knot nematode.—Perhaps, as in other tropical countries, the root-knot nematode remains active and can produce infection on susceptible plants throughout the year in the Philippine Islands. In a series of experiments in the greenhouse, susceptible crops were readily infected at any time of the year when planted in infected soil exposed to outside conditions throughout the year. Furthermore, field observations on dry farms have shown that nematode-infected plants are found at any season of the year. This is in agreement with Bessey's findings(2) that in southern Florida where the weather is warm the root-knot nematode does not have a dormant period and is active all the year round on galled roots of annual or perennial plants. In northern Florida, however, the activity of the nematodes is lessened during the winter months when the weather is colder. In the northern States their activity almost ceases, and perhaps all are killed where winters are severe. In this connection Bessey(2) further states that the nematodes are inactive when the soil temperature falls below 50° F. Godfrey,(10) in a critical study, found that below 16° C. the root knot is substantially decreased, at 10 to 12° C. infection is very rare, and at higher temperatures root knot is abundant at any temperature that the host will tolerate. As the climate is always warm in the Philippines, there is no reason to doubt that the pest can remain active and infect susceptible plants all the year round, thus making it possible for the nematode pest to be a serious problem in the Philippines once it is well established in a locality.

RELATIVE SUSCEPTIBILITY OR RESISTANCE OF TOMATO VARIETIES TO ROOT-KNOT NEMATODE

Malloch(14) in his studies on *H. radicicola* found several tomato varieties and hybrids susceptible to root knot. In our studies the American and Philippine tomatoes, which were tested in the greenhouse, were found all susceptible to this disease. In these experiments, tomato seeds were planted in 12-inch pots containing nematode-infested soil. After thirty-five days from the date of planting, the plants were lifted carefully, the roots were washed, and the percentage of infection, the average number of galls, and the average height per plant determined. Table 6 is a summary of the results obtained.

It will be seen that all the varieties are susceptible and showed a high percentage of infection. As indicated by the percentage of infection, average number of galls on the root system, and average height of plants in the variety, the relative degree of susceptibility greatly varies. Yellow Plum, Chalk's Early Jewel, June Pink, Red Cherry, Red Pear, Penn. State Earliana, Sunnybrook Earliana, The Burpee, Burpee Fordhook First, Burpee Self Pruning, and Marglobe, of the American tomatoes; and the Lemery Wild Cherry tomato, Native Pasig Nos. 1 and 5, Native San Isidro No. 1, Native Tarlac Nos. 2 and 3, Calumpit No. 2, Native Mariquina, and Native San Carlos No. 2, of the Philippine tomatoes, showed a greater degree of resistance than any of the other varieties tested. These tomatoes were but slightly affected in growth although the percentage of infection was high or, as in some cases, the galls on the roots were numerous. Plates 6 and 7 show some of the varieties that exhibit some degree of resistance and those that are susceptible. The tomato varieties, such as the Yellow Plum, Red Cherry, and other small-fruited wild types, showed only small galls, or in some instances only brownish lesions were found on the root system. No swellings were noted on the stem. The other varieties listed in the table are classed as either susceptible or very susceptible. These varieties, before the end of thirty-five days, showed many dead seedlings due to root-knot nematode infection. These varieties produced numerous galls and swellings on the stem (Plate 8). Remarks on the relative susceptibility of these tomato varieties are noted in Table 6. As far as observed, there is no variety that showed true resistance or immunity, but in general the wild small-fruited tomato showed a greater degree of resistance than the more-fleshy fruited American or Philippine tomatoes.

TABLE 6.—*Relative susceptibility of American and Philippine tomatoes to root-knot nematode, H. radicicola (Greef) Muller.*[a]

[+, Resistant; ++, moderately susceptible; +++, susceptible; ++++, very susceptible.]

Variety.	Source of seed sample.	Number of plants examined.	Infection.	Average number of galls per plant.	Average height per plant.	Susceptibility.
AMERICAN TOMATOES.			*Per cent.*		*cm.*	
Beef Steak	Burpee Seed Co	57	100	14.4	14.4	+++
Bonny Best	do	35	100	20.7	10.6	+++
Burpees Dwarf Giant	do	23	100	9.4	4.7	++++
Burpees Fordhook First	do	60	100	7.2	12.2	++
Burpees Self Pruning	do	37	100	8.3	14.9	++
Break o' Day	do	90	100	16.9	10.3	+++
Chalks Early Jewel	do	71	91.5	3.3	20.5	+
Columbia Wilt Proof	Job Wyatt & Co	50	100	11.7	10.6	+++
Columbia Wilt Proof, Maule's	Maule's Seed Co	43	100	13.8	10.7	+++
Dwarf Stone	Burpee Seed Co	56	100	20.8	4.6	++++
Early Detroit	do	28	100	12.2	5.6	++++
Golden Dwarf Champion	do	50	100	8.7	6.8	++++
Gulf State Market	do	26	100	20.2	10.2	++++
June Pink	do	63	100	12.0	20.0	+
Kilgores Special	Kilgore Seed Co	36	100	14.6	10.5	+++
Sunny Brook Earliana	Burpee Seed Co	36	100	6.0	13.1	++
Livingstone Globe	do	43	100	11.3	11.5	+++
Marglobe	United States Department of Agriculture	74	100	11.8	13.5	++
Do	Burpee Seed Co	53	100	7.5	13.0	++
Marvel	United States Department of Agriculture	42	100	16.5	5.3	++++
Norton Wilt Resistant	Steckler Seed Co	57	100	12.8	10.2	+++
Penn. State Earliana	Burpee Seed Co	63	100	14.7	16.9	+
Ponderosa, True Giant	do	42	100	12.6	12.0	+++
Red Cherry	do	57	100	4.0	20.0	+
Red Pear	do	50	48	4.3	17.0	+

Sparks Earliana	Burpee Seed Co	46	100	14.0	11.6	+++
The Burpee	do	72	100	5.8	13.0	++
Truckers Favorite	do	100	100	14.4	11.7	++++
Whole Salad	do	67	100	17.2	13.2	+++
Yellow Plum	do	45	84.4	4.1	22.0	+
PHILIPPINE TOMATOES						
Native Bureau of Plant Industry	Bureau of Plant Industry, P. I.	21	100	13.6	9.5	+++
Native Calumpit No. 2	Calumpit, Bulacan	84	100	9.7	8.6	++
Native Calumpit No. 4	do	52	100	12.0	(b)	+++
Native Mariquina	Mariquina, Rizal	22	100	11.0	10.2	++
Native Mariquina No. 1	do	32	100	16.4	9.4	++++
Native Muñoz	Muñoz, Nueva Ecija	72	100	15	(b)	+++
Native Pasig No. 1	Pasig, Rizal	97	100	7	(b)	+
Native Pasig No. 3	do	33	100	9.0	8.1	+++
Native Pasig No. 5	do	37	100	12.5	12.8	++
Native Pasig seedling	do	82	100	16.0	(b)	+++
Native San Carlos	San Carlos, Pangasinan	125	100	8.0	(b)	++
Native Santa Cruz	Santa Cruz, Laguna	72	100	16.0	7.0	++++
Native Santa Cruz No. 1	do	87	100	24.0	(b)	++++
Native San Isidro	San Isidro, Nueva Ecija	29	100	16.6	7.1	++++
Native San Isidro No. 1	do	18	100	16.0	10.8	+
Native San Isidro No. 2	do	23	100	16.0	10.3	++
Native Tarlac No. 1	Tarlac, Tarlac	80	100	26.0	(b)	++++
Native Tarlac No. 2	do	15	100	4.8	6.6	++
Native Tarlac No. 3	do	12	100	21.5	10.5	++
Native wild cherry (Lemery)	Lemery, Batangas	80	100	42.0	13.2	+

[a] Remarks on susceptibility are based on general stand of seedlings before pulling out from pots.
[b] The data on average height were lost.

TABLE 7.—*Relative susceptibility of tomato varieties obtained from different sources.*

[+, Resistant; ++, moderately susceptible; +++, susceptible; ++++, very susceptible.]

Variety.	Source of seed sample.	Plants examined.	Infection.	Average galls per plant.	Average height per plant.	Susceptibility.
			Per cent.		*cm.*	
Break o'Day	Russell Heckle Seed Co	100	100	14.0	13.1	++
Do	Burpee Seed Co	146	100	14.1	10.7	+++
Break o'Day New	Job P. Wyatt & Sons	59	100	16.9	8.1	+++
Do	United States Department of Agriculture	33	100	12.8	6.1	++
Columbia Wilt Proof, Maulis	Wm. H. Maule Seed Co	43	100	13.8	10.7	+++
Do	Job P. Wyatt Seed Co.	50	100	11.7	10.6	+++
Do	United States Department of Agriculture	25	100	8.0	7.0	+
Gulf State Market	Burpee Seed Co	26	100	20.2	10.2	++++
Do	H. G. Hastings Co	31	100	10.6	7.7	+++
Do	Wm. H. Maule Seed Co	20	100	14.0	7.4	+++
Livingstone Globe	Peter Henderson & Co	17	100	12.2	13.9	++
Do	Burpee Seed Co	43	100	11.3	11.5	+++
Do	Wm. H. Maule Seed Co	31	100	14.1	8.7	+++
Do	Ferry Morse Seed Co	18	100	7.2	7.4	+
Marglobe	United States Department of Agriculture	74	100	11.8	13.5	++
Do	Burpee Seed Co	53	100	7.5	13.0	++
Marglobe Wilt Resistant	T. W. Woods & Sons Co	69	100	12.0	10.2	++
Do	Great Northern Seed Co	50	100	15.2	10.5	+++
Do	Arthur G. Lee Seed Co	71	100	16.4	9.3	+++
Do	Ferry Morse Seed Co	32	100	14.0	9.5	+++
Do	Job P. Wyatt Seed Co.	30	100	20.7	8.8	++++
Marglobe, Starks New	Starks Bros. Seed Co	36	100	14.0	8.7	+++
Marglobe, Kilgores Bred-rite	The Kilgores Seed Co	33	100	14.1	8.8	+++
Marglobe, Wilt Resistant	Wm. H. Maule Seed Co	48	100	17.3	8.5	+++
Do	H. G. Hastings Co	38	100	12.8	8.2	+++

Marglobe	Peter Henderson & Co.	33	100	15.0	8.0	+++
Do.	Russell Heckle Seed Co.	30	100	14.4	7.0	+++
Marglobe, New	Van Anthwerp Seed Co.	26	100	10.3	6.8	+++
Marglobe	Livingstone Seed Co.	41	100	16.8	8.5	++++
Marvel (Hansings)	do.	42	100	17.8	7.8	++++
Do.	United States Department of Agriculture.	42	100	16.5	6.3	++++
Norduke Wilt Resistant	T. W. Woods Seed Co.	79	100	17.5	9.4	++++
Do.	H. G. Hastings.	44	100	20.7	8.8	++++
Do.	Job P. Wyatt & Son	39	100	7.6	7.9	++
Do.	United States Department of Agriculture.	42	100	16.3	7.7	++++
Norton Wilt Resistant	Steckler Seed Co.	57	100	12.8	10.2	+++
Do.	Job P. Wyatt & Sons	41	100	14.0	10.1	++
Do.	Great Northern Seed Co.	26	100	19.7	8.9	++++
Do.	United States Department of Agriculture.	32	100	15.3	8.2	++++
Do.	Russell Heckle	13	100	19.0	8.0	++++
Do.	Burpee Seed Co.	48	100	11.8	7.9	+++
Do.	T. W. Woods	65	100	12.0	7.7	+++
Do.	Wm. H. Maule & Sons Co.	36	100	18.3	6.7	++++
Do.	Ferry Morse Seed Co.	80	100	15.9	6.2	++++
Do.	H. G. Hastings Seed Co.	43	100	13.5	7.3	+++
Norton Improved (Starks Blight-resister)	Starks Bros. Seed Co.	40	100	16.0	8.3	+++

SUSCEPTIBILITY OR RESISTANCE OF A GIVEN TOMATO VARIETY OBTAINED FROM DIFFERENT SOURCES

In a series of trials we found no appreciable difference in the relative susceptibility or resistance to nematode infection of a given tomato variety obtained from different sources. In these experiments, few of the important American market tomatoes were tested in the same manner as in the above-mentioned experiments. The results are summarized in Table 7. Some difference in the relative susceptibility is found, however, in the different varieties, but only slight, if any, difference is noted when seeds of the same variety are obtained from different sources.

HOSTS RANGE ON HETERODERA RADICICOLA IN THE PHILIPPINES

The root-knot nematode is now known to attack over 500 species of plants. However, some plants affected vary in their degree of susceptibility. In a limited field survey and in artificial inoculation experiments in the greenhouse nearly all the common economic and weed plants in the Philippines were successfully inoculated or found naturally infected in the field with the root-knot nematode.[2] Among the economic plants affected are—

Ampalaya, *Momordica charantia* L.
Cucumber, *Cucumis sativus* L.
Melon, *Cucumis melo* L.
Patola, *Luffa acutangula* (L.) Roxb.
Upo, *Lagenaria leucantha* (Duch.) Rusby.
Watermelon, *Citrullus vulgaris* Schrad.
Tomato, *Lycopersicum esculentum* Mill.
Tobacco, *Nicotiana tabacum* L.
Eggplant, *Solanum melongena* L.
Cabbage, *Brassica oleracea* L. var. *capitata* L.
Mustard, *Brassica integrifolia* (West) O. E. Schulze.
Radish, *Raphanus sativus* L.
Sitao, *Vigna sesquipedalis* Fruw.
Sincamas, *Pachyrrhizus erosus* (L.) Urban.
Seguidilla, *Psophocarpus tetragonolobus* (L.) DC.
Beet, *Beta vulgaris* L.
Papaya, *Carica papaya* L.
Abacá, *Musa textilis* Neé.
Rice, *Oryza sativa* L.

[2] The writers are indebted to Dr. E. Quisumbing, botanist, Bureau of Science, for the identification of some of the plants.

Corn, *Zea mays* L.
Sugar cane, *Saccharum officinarum* L.
Onion, *Allium cepa* L.
Garlic, *Allium sativum* L.

Among the weed plants affected are *Ageratum conyzoides* L., *Amaranthus paniculatus* L., *Corchorus* spp., *Phaseolus lathyroides* L., *Kyllinga monocephala* Rottb., *Peperomia pellucida* (L.) HBK., *Portulaca oleracea* L., *Scoparia dulcis* L., and *Lindernia viscosa* (Willd.) Merr. Rice, corn, garlic, onion, peanuts, sincamas, and seguidilla are resistant to the root-knot nematode. Most of the weeds mentioned above are either susceptible or very susceptible to the pest. Table 8 shows the results obtained, together with an indication of the relative susceptibility of each host.

TABLE 8.—*Economic and weed plants artificially or naturally found infected with the root-knot nematode, H. radicicola (Greef) Muller, in the Philippines.*[a]

Host.		Plants examined.	Infection.	Remark on susceptibility.[d]
Common name.	Scientific name.			
ECONOMIC PLANTS			*Per cent.*	
Abacá	*Musa textilis* Neé			++
Ampalaya [b]	*Momordica charantia* L.	10	100	++++
Bean	*Phaseolus vulgaris* L.			
Canadian Wonder		5	100	++
Kentucky Wonder		3	100	++
Beet	*Beta vulgaris* L.	10	100	++++
Cabbage	*Brassica oleracea* L. var. *capitata* L.	32	100	+++
Camote [b]	*Ipomoea batatas* L.			++
Celery, "American"	*Apium graveolens* L. var. *dulce* DC.	15	100	+++
Corn	*Zea mays* L.			
Yellow Flint		22	63.6	+
White Corn		20	100	+
Lagkitan [b]		20	100	+
Cowpea	*Vigna sinensis* (L.) Savi			
New Era		20	100	++
Paayap [b]		17	100	+++
Paayap pinungo [b]		26	100	+++
Cucumber	*Cucumis sativus* L.	20	100	++++
Eggplant	*Solanum melongena* L.	25	100	++
Garlic	*Allium sativum* L.	15	100	+
Melon	*Cucumis melo* L.	10	100	++++

[a] The writers are indebted to Dr. E. Quisumbing, botanist, Bureau of Science, for the identification of the plants listed.
[b] Native common names in the Philippines.
[d] +, resistant; ++, moderately susceptible; +++, susceptible; ++++, very susceptible.

TABLE 8.—*Economic and weed plants artificially or naturally found infected with the root-knot nematode, H. radicicola (Greef) Muller, in the Philippines* [a]—Continued.

Host.		Plants examined.	Infection.	Remark on susceptibility.[d]
Common name.	Scientific name.			
ECOMOMIC PLANTS—Ctd.			*Per cent.*	
Mungo [b]	*Phaseolus radiatus* L.	10	100	++
Mustard	*Brassica integrifolia* (West) O. E. Schulz	25	100	+++
Okra	*Abelmoschus esculentus* (L.) Moench	5	100	++++
Onion	*Allium cepa* L.	10	50	+
Papaya	*Carica papaya* L.	22	100	++
Patola [b]	*Luffa acutangula* Roxb.	4	100	+++
Peanut	*Arachis hypogaea* L.	15	100	+
Upo [b]	*Lagenaria leuchantha* (Duch.) Rusby	10	100	++++
Radish	*Raphanus sativus* L.	25	100	+++
Rice	*Oryza sativa* L.			
Dumali [b] (upland variety)		40	25	+
Seguidilla [b]	*Psophocarpus tetragonolobus* (L.) DC	7	87.7	+
Sincamas [b]	*Pachyrrhizus erosus* (L.) Urban	20	50	+
Pangasinan seeds		9	77.8	+
Bulacan seeds		10	70.0	+
Bureau of Plant Industry seeds		15	66.7	+
La Union seeds		11	100	+
Sitao [b]	*Vigna sesquipedalis* Fruw.	5	100	++++
Squash	*Cucurbita maxima* Duchesne	25	100	++++
Sugar cane	*Saccharum officinarum* L.			+
Tomato [c]	*Lycopersicum esculentum* Mill.	75		++++
Tobacco	*Nicotiana tabacum* L.			
Batec [b]		25	100	++++
Bansoroy [b]		30	100	++++
Viscayan [b]		25	100	++++
Watermelon	*Citrullus vulgaris* Schrad.	15	100	++++
WEED PLANTS				
Damong-pallas [b]	*Ageratum conyzoides* L.	5	100	+
Kalunai [b]	*Amaranthus paniculatus* L.	15	100	++++
Saluyot [b]	*Corchorus* sp.	25	100	+
	Phaseolus lathyroides L.	10	100	++++
Mutha [b]	*Kyllinga monocephala* Rottb.	5	100	+
Olasiman ihalas [b]	*Peperomia pellucida* (L.) HBK.	5	100	+++
Ulasiman [b]	*Portulaca oleracea* L.	10	100	++
Sampalokan [b]	*Scoparia dulcis* L.	5	100	++
	Lindernia viscosa (Willd.) Merr.	5	100	++
"Tagulinai" [b]	*Vernonia cinerea* (L.) Less.	6	100	++

[a] The writers are indebted to Dr. E. Quisumbing, botanist, Bureau of Science for the identification of the plants listed.

[b] Native common names in the Philippines.

[c] For tomato varieties refer to Table 7.

[d] +, resistant; ++, moderately susceptible; +++, susceptible; ++++, very susceptible.

DISCUSSION AND SUGGESTION FOR CONTROL

In the present investigation, experiments on control measures applicable to Philippine conditions have not been undertaken. As the disease attacks the underground parts of the plant, any protective material applied on the roots will be useless. The control of nematodes must, therefore, be directed against the eradication of the pest from the soil, and this can only be accomplished by sterilizing the soil with steam or chemicals, or by rotation of crops to starve out the organism. Another method is the selection and development of resistant strains.

The control of the root knot has been well studied in the United States and effective control measures have been recommended. Because of the high cost of materials, these measures are of a limited practical application, so much so that even the truck farmers in the United States cannot well afford the expenses entailed in the purchase of the chemicals and machinery used. Under Philippine farming conditions, while there are measures which cannot be recommended, other workable control and preventive measures can be tried. We will mention and discuss some of these well-known control measures.

Soil sterilization by steam.—Sterilization by steam is the most practical method of control in soils in greenhouse and seed beds or in small plots. It is accomplished by passing steam through perforated pipes at a depth of one foot below the surface for one or two hours, depending upon the steam pressure and type of soil.(9) Another way of using steam is the "inverted pan method," in which an iron or a wooden pan of convenient size is used. The pan is inverted over the bed, the edges thrust into the soil, and steam under pressure forced into the pan through a special inlet for from one-half to two hours.(9) Small quantities of soil or soil in pots or flats can be readily sterilized in an autoclave for two to five hours or in specially constructed vaults heated to 110° C. or more for two or more hours, the length of time depending upon the steam pressure. Pouring hot water directly on pots, flats, and benches at the rate of 7 gallons per cubic foot is also satisfactory,(4) and although its application is limited, it is effective in ridding the soil of nematodes where steam is not available.

Soil sterilization by chemicals.—The use of chemicals has been tried by various workers, but in almost all cases the chemicals found to be effective are too expensive for practical use in the field. Durez(7) in 1917 recommended 200 pounds of

sodium cyanamide (NaCn) per acre dissolved in water and applied at the rate of $\frac{1}{3}$ gallon per square foot of soil, followed by a second treatment a week later; this gave perfect control in the greenhouse. Watson(19) in 1921 likewise recommended the use of dry powdered calcium cyanamide at the rate of 600 to 800 pounds per acre carried down to a depth of about 18 inches by irrigation water. After this treatment, ammonium sulphate at the rate of 900 to 1,200 pounds to the acre is applied. Because of the high cost of these chemicals for field operations, their use is not popular with farmers in the United States. These chemicals have been adopted for seed beds and small truck patches where intensive cultivation of a susceptible crop is practiced. McClintock(15) and Stone and Smith(18) tried other chemicals such as carbon bisulphide, corrosive sublimate, calcium carbide, ammonium sulphate, formaline, nicotine, benzene, kerosene, magnesium sulphate, common salt, potassium nitrate, magnesium sulphate, calcium sulphate, kainit, sodium nitrate, and lime, but no satisfactory results were obtained.

Rotation of crops.—Rotation of crops for two or three years is generally recommended as an effective means of control.(8, 9, 20) The crops known to be resistant or immune to root-knot nematode in the United States are the true grasses (crabgrass and Bermuda grass), most varieties of corn, wheat, rye, and some varieties of oats, velvet beans, and beggar weed, and the iron and Bradham cowpeas. Peanuts, onions, parships, strawberries, and turnips are slightly affected. These crops have been recommended as rotation crops before planting the choice susceptible crops. Our studies have shown that rice, corn, onion, garlic, peanuts, sincamas, and seguidillas are also highly resistant to the root-knot nematode, and these should be recommended as rotation crops in the Philippines if the resistant foreign plants cannot be grown successfully. In order to shorten the time of rotation Weber and Ramsey(20) recommended a summer fallow, in which the land is plowed, cultivated, and kept bare of vegetation all summer. This method is quite severe on soil fertility, but he suggested a modification in which velvet beans could be planted and kept well cultivated as a cover crop. Under Philippine conditions, and especially in regions where there is a distinct dry hot season, the summer fallow method suggested by Weber might be adopted if two or three years of crop rotation cannot be followed.

Other control measures.—The selection and development of resistant varieties to control nematode infection offer a great field for investigation and would be the best method of control if resistant strains could be developed. Other methods, such as flooding the infected soil for two to four months, or plowing infested lands during the dry hot months in order to desiccate the nematode, are worthy of consideration. As important as are the methods above enumerated, the eradication of weeds, a clean culture, and the prevention of a promiscuous introduction of the pest from one locality to another through nursery stocks, or dirt which accompany propagating seedlings, should not be overlooked. The spread of the disease from one field to another should be avoided by proper precaution in the use of irrigation water, tools, plows, and work animals. A man's shoes should be cleaned or disinfected before he moves from an infested field to one free from the nematode.

SUMMARY

1. The root-knot nematode, *Heterodera radicicola,* is common and widespread in the Philippines, but thus far it is not so serious as to limit the production of the important truck and field crops.

2. Characteristic symptoms of plants infected with nematode are described.

3. The root knot found on tomato, tobacco, and other plants in the Philippines is caused by the well-known *Heterodera radicicola* (Greef) Muller, and so far no evidence of biologic strains of *H. radicicola* has been found.

4. Susceptible plants are readily infected with root-knot disease when grown in nematode-infested soil.

5. Root-knot infection was obtained at any soil moisture capable of supporting plant growth. A high percentage of infection was obtained even when the soil was saturated with water, or kept relatively dry.

6. Flooding of infected soil for thirty-five to forty days did not kill all the nematodes but the percentage of infection was greatly decreased.

7. Experiments showed that nematode-infested soil may be freed from the pest by air desiccation for twenty-five to thirty-five days. The length of time necessary to free the infested soil seems to depend on the initial moisture content of the soil.

8. The larvæ are very sensitive and are killed within ten to fifteen minutes of drying in the laboratory. The larvæ of the younger stage died much quicker than those of the older stages. Young larvæ were killed after three minutes of complete drying in the laboratory.

9. The larvæ suspended in water or kept in moist condition were found to survive much longer without food. Living larvæ were still observed after thirty-five days in water suspension under laboratory conditions.

10. Under Philippine conditions the root-knot nematode remains active all the year round. Root-knot infection can be produced at any time of the year from infected soil exposed to outside condition throughout the season.

11. All the American and Philippine tomatoes tested are susceptible to the root-knot nematode. Some varieties showed more resistance than the others, but the wild, small-fruited type and a few of the Philippine tomatoes are in general more resistant than the fleshy-fruited varieties.

12. The source of tomato seeds of a certain variety did not show any difference in their relative susceptibility.

13. Several common economic crops and weed plants in the Philippines are found susceptible to the root-knot nematode, and reported here for the first time. Rice, corn, garlic, onions, sincamas and seguidilla are found to be highly resistant to root-knot nematode.

14. No experiments on control measures were undertaken, but the already known control measures are suggested and discussed.

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ILLUSTRATIONS

PLATE 1

Root-knot symptoms on the roots of tomato and a weed, *Phaseolus lathyroides*, which are naturally infected in the field with the root-knot nematode.

FIG. 1. Mature tomato plant.
2. *Phaseolus lathyroides* Linn.

PLATE 2

Root-knot symptoms on the roots of tobacco, sitao, and garden beet plants.

FIG. 1. Tobacco.
2. Sitao.
3. Garden beet.

PLATE 3

Tobacco plant artificially infected in the greenhouse with the root-knot nematode, fifty days after inoculation. The main and secondary roots show swelling.

PLATE 4

Tobacco plants naturally infected in the field with the root-knot nematode. These plants were obtained from San Jacinto, Pangasinan Province, Luzon.

PLATE 5

Morphological stages of the root-knot nematode, *H. radicicola* (Greef) Muller. With the exception of the mature male nematode, all drawings were made with the aid of the camera lucida.

FIG. 1. Mature male, drawn under a low-power lens; about × 170.
2. Mature female, about × 73.
3. Egg sack, about × 73.
4. Various stages, *a*, *b*, *c*, *d*, in the development of the egg, about × 280.
5. Young larvæ, about × 170.

FIGS. 6 to 10. Various stages in the development of the female nematode. Fig. 10, the mature female, is about × 70; the others are about × 100.

PLATE 6

Photographs showing the relative susceptibility or resistance of tomatoes to the root-knot nematode; forty days after planting, about one-fifth natural size.

FIG. 1. Break o'Day.
2. Chalk's Early Jewel.
3. Dwarf Stone.
4. True Giant Ponderosa.
5. Yellow Plum variety.

PLATE 7

Photograph of tomato varieties, resistant and very susceptible to the root-knot nematode. Ten plants; forty days from date of planting, about one-third natural size.

FIG. 1. Break o'Day.
2. Chalk's Early Jewel.
3. Dwarf Stone.

PLATE 8

The two susceptible tomato varieties shown in Plate 7, slightly less than natural size.

FIG. 1. Break o'Day.
2. Dwarf Stone.

1 2

PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

PLATE 5.

1 2

3

4 5

PLATE 6.

PLATE 7.

B25
B12
METRIC SYSTEM
INCHES
ARTHUR H. THOMAS CO.
PHILADELPHIA
1
2

PLATE 8.

THE CATHARTIC EFFECTS IN MAN OF THE LEAVES OF WIKSTROEMIA OVATA MEYER (SALAGO LEAVES)

By Faustino Garcia

Of the Department of Pharmacology, College of Medicine University of the Philippines, Manila

Wikstroemia ovata Meyer(1) is a plant known locally by the name of "salago" in Tagalog; "malasampaga" in Pampango; "arandon" in Ilocano; and "dapnit" or "suka" in Bontoc. It is found growing in thickets and primary or secondary forests at low and medium altitudes in Luzon, Negros, and Mindanao. In Luzon, it is scattered in Bontoc, Benguet, Bulacan, Pampanga, Bataan, Rizal, Laguna, Batangas, and Sorsogon Provinces. Blanco(2) described it under the names *Daphne phoetida* and *D. indica.* His descriptions of the plants and their uses are as follows:

Daphne Phoetida. Dafne fetida. Tronco con las ramas medio ahorquilladas. Hojas opuestas, algo acorazonadas aovadas, muy aguzadas enteras y lampiñas. Peciolo cortísimo, y arriba con un canal alado. Flores terminales, en umbela simple. Involucro de la umbela, ninguno. Cal. ninguno, a no ser que se quiera llamar caliz a la corola. Cor. tubulada carnosa, tosca, de color verdecino, y el limbo con cuatro lacinias. Estam. ocho, fijos en las paredes de la corola, y colocados en dos series, esto es, cuatro en la garganta, y los otros alternos más abajo. Filam. cortísimos. Ant. asaeteadas. Germen cilíndrico, rodeado de dos escamas pequeñas hendidas en los extremos. Estilo ninguno. Estigma globoso. Baya superior, con una semilla globosa, con la corteza coriacea y delgada. = Estos arbolitos menores que el brazo, se elevan a la altura de nueve pies. Los indios generalmente conocen el *Salago,* aunque algunas veces enseñan una cosa por otra. Son comunes en las playas del mar. Su corteza es tenacísima, y he visto que se puede hacer facilmente papel de ella. Para esto basta hecharla en agua, y sacarla al día siguiente o a los dos días. Se vuelva al agua después de seca, y se la pisa bien con los pies, y se deja secar al sol. Esta operación se continuará por algunos días, hasta que el material tome la blancura que se desea. Aunque creo no ser necesario, por lo que yo he experimentado, molerle en molinos, siempre será bueno hacerlo, para lograr una mayor y más perfecta división. Este tiene el color ceniciento, y no se podrá blanquear más. Las hojas se usan con efectos muy satisfactorios para purgar: y sobre esto tanto en el número de ellas, como

en el modo de arrancarlas, forman los indios varias supersticiones. Las hojas según ellos, no se pueden tomar sino en numero impar: y si se arrancan hacia abajo, toda la operación será por abajo: y por vómito, si se arrancan tirando hacia arriba: se toman en peso de doce granos, o la cuarta parte de una dracma, mezclados sus polvos en el chocolate, o con miga de pan, y se hacen píldoras. Los indios de Tanawan, creen que se alivia la papera enfermedad común en aquel y algun otro Pueblo de Batangas, ciñéndola con una cinta de la corteza del arbolito. Flr. en Jun. T. Salago, Malasampaga.

Daphne Indica. Dafne de indias., Hojas opuestas aovadas alargadas, aguzadas enteras y lampiñas. Peciolos cortísimos con canal un poco alado. Flores terminales en umbela, Cor. de color amarillo, de figura de embudo, arqueada, con cuatro lacinias lanceoladas. Estam. como en la especie anterior. Anteras encarnadas. Estilo corto. Lo demás como en la especie anterior. = Arbolitos conocidos en Bulacán, y cuyas hojas sirven para purgar, como dije antes: las flores no son feas como las del anterior salago. T. Salago, Malasampaga.

According to Merrill(3) both species described by Blanco, under the names *Daphne foetida* and *D. indica*, were manifestly *Wikstroemia ovata* Meyer.

From the book of Blanco it can be seen that this plant "salago," or *Wikstroemia ovata*, has been used by the natives as a purgative even before 1837. According to Juan Tolentino, of Mesulao, Calumpit, Bulacan, the first one who mentioned the cathartic property of the plant to me, the leaves are a strong purgative when chewed and swallowed and one bowel movement is produced for every leaf taken. All other properties cited by Blanco were known to him. To verify and study more about the cathartic property of the leaves of *Wikstroemia ovata*, commonly known as salago, this investigation was carried out. The specimens that were used for investigation were supplied me partly by Mr. Juan Tolentino and mainly by Dr. E. Quisumbing and were identified as *Wikstroemia ovata* by Drs. Leon M. Guerrero and E. Quisumbing, of the Bureau of Science.

THE EFFECTS OF "SALAGO" LEAVES IN MAN

The dose advised by Blanco as a cathartic is from 0.8 to 1.0 gram of the dried powdered leaves taken mixed with chocolate or bread. As the weight of a large matured green leaf is about 0.15 to 0.18 gram and, on drying, it is generally reduced to one-half or one-third of its weight, five green leaves would be within the dose recommended by Blanco. Believing that five leaves or less when chewed and swallowed would produce only catharsis

in man I tried them on volunteers. Three subjects presented themselves, and to each of them I gave five green leaves which they chewed and swallowed, followed by a glass of water. The expected catharsis was produced, but the tenesmus and other side effects were so great that none of the three would take the leaves again. In subsequent experiments, the dose was reduced; and with the help of medical students, who at the beginning were skeptical about my statement with reference to the cathartic property of the leaves, I was able to obtain results of different doses. The effects were qualitatively similar but quantitatively different depending upon the dosage. The following case may serve as an illustration of the usual effects of the green leaves:

G. N. D., adult medical student, weighing 45 kilograms; habitual bowel movement every second or third day. Bowel movement the morning of January 8, 1932, and none the next morning. January 9, 1932, at 10.11 a. m., he chewed and swallowed four green leaves with a total weight of 0.4 gram followed by a glass of water.

10.16 a. m., warm and biting sensation in the mouth and throat and slight numbness at the tip of the tongue, which increased in intensity to a burning, sharp, biting taste with some numbness two hours after, persisted and then gradually diminished until it disappeared at about 6.10 p. m. The numbness persisted until the next morning.

11.25 a. m., slight stomachache with perspiration. Eructation and flatus occurred, gurgling sounds; uneasiness and bodily weakness were felt. These symptoms continued at intervals throughout the afternoon.

12 noon, first bowel movement; stool composed of small, pill-like, solid fæces.

12.20 p. m., took his usual luncheon.

12.35 p. m., second bowel movement; a large amount of watery stool, preceded by slight tenesmus and sensation of heat in the anus during defecation.

1.25 p. m., third bowel movement, greenish watery stool, less in amount than in the second bowel movement. Slight tenesmus was present.

2.15 p. m. fourth bowel movement, similar to stool in the third but scanty in amount.

6.30 p. m., slight stomachache and accompanying symptoms.

7.20 p. m., took his dinner.

7.40 p. m., felt all right again, the same as before taking the leaves.

January 10, 1932, no bowel movement.

January 11, 1932, one bowel movement of soft but formed stool.

January 12, 1932, no bowel movement.

Similar effects were obtained in other cases with more or less intensity depending upon the dose. For convenience of discussion the cathartic effects of different doses of green leaves in twelve subjects are given in Table 1.

TABLE 1.—*Cathartic effects of green salago leaves in man, chewed and swallowed.*

Subject's initials.	Weight of subject.	Dose.	Matured, large and medium-sized, green leaves.	Time of administration.	
	kg.	*g.*		*a. m.*	*p. m.*
F. G	56	0.15	1	-----	1.29
A. A. P	60	0.28	2	10.50	----
R. J. B	46	0.30	2	10.46	----
A. S	57	0.30	2	11.47	----
J. T	52	0.40	4	10.10	----
G. N. D	45	0.40	4	10.11	----
J. N. A	50	0.50	3	10.49	----
M. G. B	81	0.62	4	10.43	----
J. C	65	0.70	4	10.50	----
J. K. J	75	0.80	5	-----	1.53
L. P	60	0.85	5	-----	1.25
P. S	51	0.85	5	-----	1.42

Subject's initials.	Time of action.		Bowel movements.	Duration of action. Time between first and last cathartic bowel movement.		Remarks. All leaves pulled upward on picking.
	Hrs.	*mins.*		*Hrs.*	*mins.*	
F. G	17	41	1	--	--	In addition one bowel movement at usual time.
A. A. P	6	10	2	13	00	
R. J. B	5	6	1	--	--	
A. S	1	28	1	--	--	
J. T	2	10	3	5	00	
G. N. D	1	49	4	2	15	
J. N. A	1	41	3	1	30	
M. G. B	1	32	3	1	00	
J. C	1	50	5	5	50	Very slight blood stain and much mucus in stool.
J. K. J	7	37	4	10	00	
L. P	6	15	5	13	50	
P. S	1	48	5	14	00	Vomited twice.

A glance at Table 1 shows that increasing the dosage increased the number of bowel movements produced, but apparently there is no tendency to produce one bowel movement for every 0.15 gram of the leaves. The relation is more in favor of almost one bowel movement for every leaf taken. This relation would naturally be affected by the weight or resistance of the patient. For instance, there was one whose weight was 81 kilograms who chewed and swallowed four large green leaves weighing 0.62 gram and produced only three bowel movements.

On the other hand one whose weight was 45 kilograms, chewing the same number of leaves but with less weight or 0.4 gram produced four bowel movements. Considering the dose by the leaves it will be seen that in the three subjects who took five leaves, five bowel movements were produced in two and four in one; of the four who chewed four leaves of different sizes, two produced three bowel movements, one produced four, and one produced five bowel movements; the average was almost four bowel movements; one took three leaves and produced three bowel movements; of three who chewed two leaves, one produced two bowel movements and two one bowel movement plus the ordinary bowel movement the next morning. With one leaf the effect was delayed and seemed to increase only the normal bowel movement by one at the usual time of defecation. For practical purposes, therefore, it may be stated that one bowel movement was produced for every large green leaf taken. The time of action has some relation to the time of administration. With three to four leaves taken one to two hours before a meal, the usual time of response was from one and one-half to two hours. With smaller doses, one to two leaves, the time of cathartic response was delayed from five to seventeen hours. Taken one to two hours after a heavy meal, the time of action was delayed from two to seven hours even with large doses. The duration of the cathartic action varied with different doses. In general, doses of three to four leaves with weights from 0.4 to 0.6 gram produced catharsis within from one to five hours, and with larger doses the duration was prolonged from ten to fourteen hours. The stools were all watery except the first bowel movement, which might be formed at the beginning and soft or watery at the last part. The quantity was generally much in the first and second stools and scanty at the later bowel movements.

Side effects.—Disagreeable abdominal sensations, similar to those of hunger, occurred at intervals and caused slight perspiration, bodily weakness, and uneasiness rather than pain, and slight tenesmus just before defecation; these were frequent manifestations in all cases. These effects and those produced on the mouth and throat were the side effects disliked by all who took the leaves. The effects on the mouth and throat were qualitatively similar in all cases even with small doses but quantitatively different, and the quantitative variation was due probably to the length of mastication one employed before swallowing.

These effects consisted of warm to burning sensation in the mouth and throat, sharp, biting or pungent taste, and some feeling of numbness, especially at the tip of the tongue. All were present from a few minutes after chewing, and gradually became more intense until two to three hours after, gradually subsided and disappeared in ten to eighteen hours after mastication. With large doses, one patient vomited twice during the height of catharsis and in another a slight stain of blood and much mucus were observed in the first bowel movement but were absent in subsequent bowel movements. The catharsis produced by large doses was so great that one of the subjects could not delay defecation long enough to reach the toilet.

It is not true, as indicated in the literature, that leaves pulled upward in picking will cause vomiting. All the leaves used in my experiments were picked by being pulled upward and still all the effects were catharsis, and only in one instance a large dose produced both vomiting and catharsis.

CORRECTION OF SIDE EFFECTS

To avoid the disagreeable effects on the mouth, the leaves were sliced and placed in capsules before administration. To correct the mild colic or abdominal pain belladonna powder was administered at the same time. Administration of the leaves in capsules completely eliminated the disagreeable effects on the mouth and throat, but the cathartic actions were modified, as shown in Table 2.

When the leaves were cut in big slices 2 millimeters or more wide, placed in capsules, and then administered by mouth, the cathartic effects were not manifested with doses that would surely have been effective if the leaves had been chewed and swallowed as indicated in previous experiments and in the control shown in Table 2. If the leaves, however, were cut into fine slices 1 millimeter or less in width, placed in capsules, and similarly administered by mouth some modified cathartic effects were produced. The cathartic effects were less in general compared with similar doses of leaves that were chewed and swallowed. The time of action was delayed in nearly all subjects who took the leaves with belladonna powder and the duration of its action was generally prolonged in several cases. The number of bowel movements produced has no relation to the

doses given as in the cases of those who chewed and swallowed the leaves. Belladonna powder did not lessen the abdominal pain that usually preceded or accompanied the cathartic effects of the leaves.

TABLE 2.—*Influence of capsules, size of slices, and belladonna powder on the effects of salago leaves.*

Subject's initials.	Weight of subject.	Dose.	Leaves.	Time of administration.
	kg.	*g.*		*a. m.*
M. B.	58	0.42	3	9.35
J. B.	45	0.53	4	9.40
L. S.	57	0.53	4	9.43
F. G.	56	0.37	3	11.01
J. B.	45	0.35	3	9.36
M. B.	58	0.4	3	9.37
V. G. B.	53	0.26	2	11.44
J. C.	65	0.3	2	11.40
J. R. A.	48	0.4	3	11.44
D. J. A.	50	0.48	3	11.42
J. A.	48	0.45	3	11.03
M. B.	58	0.52	4	11.30
A. J.	47	0.60	4	11.02
V. A. F.	62	0.65	4	11.05

Subject's initials.	Time of action.		Bowel movements.	Duration of action.		Remarks.
	Hrs.	*mins.*		*Hrs.*	*mins.*	
M. B.	----	----	0	----	----	Big slices in capsules.
J. B.	----	----	0	----	----	Do.
L. S.	----	----	0	----	----	Do.
F. G.	----	----	0	----	----	Big slices in capsules with belladonna.
J. B.	1	57	2	8	31	Leaves chewed and swallowed.
M. B.	4	01	3	3	40	Do.
V. G. B.	----	----	----	----	----	Fine slices in capsules.
J. C.	1	50	2	2	30	Do.
J. R. A.	1	56	4	17	30	Do.
D. J. A.	3	59	2	4	29	Do.
J. A.	16	0	3	0	50	Fine slices in capsules with belladonna powder. Abdominal pain present as without belladonna.
M. B.	13	0	2	10	30	Fine slices in capsules with belladonna powder. Abdominal pain present as without belladonna. Stomach pain, sensation of fullness one hour and five hours after.
A. J.	20	58	1	----	----	Abdominal pain.
V. A. F.	10	10	3	9	4	Abdominal pain, eructation, and headache.

IS THE ACTIVE PRINCIPLE PRESERVED ON DRYING?

"Salago" leaves were allowed to dry in the laboratory room and the weight was found to be reduced from 0.28 gram of the green leaves to 0.1 gram of the dried. These dried leaves were used in the experiments by chewing and swallowing, at different intervals, and the results obtained are given in Table 3.

TABLE 3.—*Effects in man of dried "salago" leaves chewed and swallowed.*

Subject's initials.	Weight.	Time of drying.	Dose.	Leaves.	Time of administration.	Time of action.		Bowel movements within 18 hours after administration.	Remarks.
	kg.	*Days.*	*g.*		*a. m.*	*Hrs.*	*mins.*		
J. B_____	45	15	0.15	3	9.40	3	0	1	Diarrhœic stool; usual bowel movement next day; usual sensation in mouth.
A. P_____	62	15	0.18	3	9.40	0	35	2	Diarrhœic stool; usual sensation in mouth.
M. B_____	58	15	0.25	5	9.41	3	2	3	Watery stool.
D. J. A__	50	22	0.1	3	10.39	----	----	0	Only bitting and warm sensation in mouth.
S. A. D___	56	22	0.2	4	10.42	2	21	4	Watery stool.
F. G_____	57	44	0.27	5	10.11	2	56	1	Watery stool; usual bowel movement next day and warm sensation in mouth.
		Years.							
F. C_____	52	2	0.14	3	10.50	----	----	0	Slight warm sensation in mouth that lasted about one-half to 2 hours.
A. C. T___	53	2	0.2	4	10.10	----	----	0	Do.
F. L_____	45	2	0.2	4	10.10	----	----	0	Do

Judging from the data in Table 3, one will conclude that the activity of the leaves is decreased or lost upon drying. Three to five leaves, which ordinarily when taken fresh moved the bowels from three to five times, produced fewer or no movements, depending upon the length of time of drying. There was slight or no diminution with fifteen to twenty-two days drying, distinct reduction with forty-four days, and total loss of activity in causing catharsis with 2-year-old dried

leaves with doses of 0.2 gram equivalent to at least 0.58 gram of green leaves. The effect on the mouth was still present but greatly reduced, indicating that the active principle, at least that causing warm sensation in the mouth, was not completely lost on two years drying, but what was left in the dose given was not sufficient to cause catharsis. On account of the variable effects of the dried leaves and in view of the fact that it was difficult to obtain always fresh material for experiments when needed, the chemical and pharmacodynamic studies were rendered difficult. Studies are being continued along these lines.

SUMMARY AND CONCLUSIONS

1. The green leaves of *Wikstroemia ovata* Meyer (salago leaves) when chewed and swallowed produced varying degrees of catharsis, depending upon the number of leaves taken. In general it may be stated that every large leaf caused one watery bowel movement.

2. One to two large leaves of "salago" chewed and swallowed, caused generally one to two laxative or purgative effects, and three to four leaves, weighing from 0.4 to 0.6 gram produced three to four watery stools apparently drastic in character. The therapeutic cathartic doses may be placed between one and four leaves, inclusive, or 0.15 to 0.65 gram. Five large leaves, weighing at least 0.8 gram, produced not only catharsis but in some cases vomiting and other pronounced side effects.

3. The side effects noted were the effects on the mouth and throat when leaves were masticated and consisted of a warm to burning sensation and pungent and biting taste, mingled, later, with numbness. These effects were intensified in two to three hours and then gradually subsided in ten to sixteen hours. Eructation and flatus frequently occurred in one to two hours or more before the cathartic effect. Sensation of fullness in the abdomen, slight abdominal pain occurring at intervals, mild tenesmus, perspiration, and bodily weakness were the frequent complaints of the subjects.

4. The undesirable effects on the mouth and throat could be avoided by administration of the leaves in capsules, but in this form of administration the cathartic effects were uncertain. The abdominal pain and tenesmus were apparently not mitigated by the administration of therapeutic dose of belladonna.

5. The cathartic active principle was partly or mainly lost during desiccation at room temperature and in keeping the

leaves in the dried form. Some of it was still left after two years, enough to give distinctly the usual effect on the mouth when chewed, but not enough to produce catharsis as when freshly taken. The deterioration of the active principle in drying and keeping rendered the chemical and pharmacodynamic studies difficult on account of the lack of fresh material when needed.

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NEUE CASSIDINEN VON DEN PHILIPPINEN-INSELN (COLEOPTERA; CHRYSOMELIDÆ)

Von Franz Spaeth

Vienna, Austria

PRIOPTERA LATISSIMA Wagen. POLILLOENSIS subsp. nov.

Auf Polillo hat Herr W. Schultze ein Männchen einer *Prioptera* gesammelt, das ich für eine sehr interessante Abart der *P. latissima* halte. Auf jeder Flügeldecke sind vier schwarze Flecke: der 1. rund und ziemlich gross, vorne neben der Naht and Höckerstelle, schräg vor dem grossen Hauptgrübchen; bei der Nominatform fehlt er oder ist viel kleiner; ich habe schon seinerzeit in meiner Revision der Gattung *Prioptera* [1] aufmerksam gemacht, dass im Gegensatz zu anderen Arten dieser Fleck bei *latissima* nicht in, sondern vor dem Hauptgrübchen steht, und es ist daher ein Beweis für die Zugehörigkeit der vorliegenden Unterform zu *latissima*, dass dieser Fleck auch bei ihr hauptsächlich vor und nur mit einem kleinen Teil in der Grube steht. Der 2. Fleck ist kleiner, mehr quer, genau hinter dem ersten auf dem Abfall; dieser Fleck fehlt bei der Nominatform ganz. Der 3. ist genau wie bei dieser gebildet und gestellt: strichfömig, halb verloschen, aussen vom dritten Grübchen, im drittletzten Zwischenraum; der 4. ist eine bis an den Aussenrand reichende Querbinde hinter der Mitte des Seitendaches, eine Vergrösserung des dort bei der Nominatform vorkommenden, kleinen Fleckes.

In der Körperform und Skulptur, insbesonders der vorderen, tiefen Ausrandung des Halsschildes und der feinen, gleichmässigen Punktierung der Flügeldecken, stimmt *polilloensis* ganz mit der Nominatform überein.[2]

[1] Philip. Journ. Sci. 28 (1925) 414. Dort steht auf p. 414 infolge eines Korrekturfehlers in der 15. und 24. Zeile von oben irrtümlich statt "und zwar"-"und zuweilen;" derselbe Fehler ist auch p. 407, 11. Zeile von unten.

[2] Ich benütze die Gelegenheit der vorstehenden Publikation in The Philippine Journal of Science, die dort erschienene obige Revision der Gattung *Prioptera* durch die folgende Beschreibung einer neuen *Prioptera* zu ergänzen.

PRIOPTERA LAOTICA sp. nov.

Obtriangularis, modice convexa, nitida, albidoflava, subtus cum antennis et pedibus laete testacea, prosterno, pectore medio, capite, antennarum articulo ultimo, denique vitta transversa pone medium protecti nigris; prothorax laevis, antice late, minus profunde emarginatus; elytra prothorace basi haud latiora, lateribus ultra medium sensim rotundatoampliata, disco non gibbosa, mediocriter et subregulariter punctatostriata, protecto laevi.

Mas magis rotundatus, brevior, antennis longioribus, articulo ultimo longiore et crassiore, apice ciliato; long. 8, lat. 7 mm.

Femina minus rotundata, antennis brevioribus, articulo ultimo breviore; long. 8, lat. 7 mm.

Tonkin, Lao-Kai.

Mässig gewölbt, oben stark glänzend, weisslichgelb, unten gesättigter gelb; das Prosternum, die Mitte der Brust, der Kopf, das letzte Fühlerglied und eine schmale Querbinde hinter der Mitte des Seitendaches sind schwarz. Die grösste Breite liegt kurz vor ihr, von wo die Flügeldecken zur Basis verengt sind, wo sie die gleiche Breite wie der Halsschild haben. *Prioptera laotica* ist nur mit *prognata* m. von Darjeeling nahe verwandt, hat die ganz gleiche Körperform und Zeichnung und unterscheidet sich von ihr durch ihr breiteres Seitendach, mehr gerundet verlaufende Seiten (zum Vergleiche dient das Weibchen, da ich von *prognata* nur dieses kenne) etwas schlankere, dünnere Fühler, besonders aber durch die zwar auch in unregelmässigen, verworrenen Streifen, aber viel feiner, weniger runzelig und dicht punktierten Flügeldecken; bei *prognata* sind die Zwischenräume schmäler als die Streifen, zum Teil herausgewölbt und die Punkte der mittleren Streifen (3 bis 7) greifen, besonders in den Grübchen, ineinander über, bei *laotica* ist letzteres nicht der Fall, die Punkte sind feiner, und stehen weniger dicht, die Zwischenräume sind vielmals breiter und es können die Streifen überall, auch in den Grübchen, getrennt verfolgt werden. Die Spitze der Epipleuren ist in beiden Geschlechtern fein und lang behaart.

Wollte man *P. laotica* nach meiner Tabelle[3] bestimmen, so würde man auf *prognata* gelangen.

Vier Stücke sind in meiner Sammlung.

LACCOPTERA SCHULTZEI sp. nov.

Subtriangularis, subnitida, gibbosa; subtus flavotestacea, pectore nigro, antennis basi flavotestaceis, articulis 4 ultimis nigres-

[3] Philip. Journ. Sci. 28 (1925) 381.

centibus, supra testacea, protecto prothoracis dilutiore, protecto elytrorum hyalino, albido, ramis duobus utrinque apiceque testaceis, gibbere maculisque nonnulis disci indistinctis picescentibus; prothorax ad basim haud angustatus, disco sat profunde longitudinaliter aciculato; elytra prothorace fere duplo latiora, humeris subacutis, gibbere sat alto, antice posticeque sat profunde emarginato, disco subregulariter punctato-striato, interstitiis alternis, imprimis secundo subcarinatis, protecto in ramis profundius rugoso-punctato; unguiculi intus tantum pectinati; long. 10, lat. 9 mm.

MINDORO, Abra de Ilog (*W. Schultze*).

Von fast dreieckigem Umriss, in den Schultern am breitesten, von hier nach hinten fast geradlinig verengt, beinahe zugespitzt, nur das äusserste Ende verrundet. Unten braungelb, die Brust (ohne Seitenteile) schwarz; letztere Farbe haben auch die vier letzten Fühlerglieder. Oberseite gelbbraun, das Vordach des Halsschildes und das Seitendach durchscheinend weissgelb, letzteres mit zwei breiten hellbraunen Randästen, einer ganz an der Basis, der andere hinter der Mitte; die Höckerstelle ist etwas dunkler; auf den höheren (primären) Zwischenräumen sind hinter der Mitte einige braune Flecken.

Halsschild nur halb so breit als die Basis der Flügeldecken, vorne flach gerundet, die Seiten nach hinten kaum verengt, die Scheibe mit ziemlich tiefen und langen Längsrissen, das Vordach mit einigen, unscharfen Falten. Flügeldecken mit wenig vortretenden, aber seitlich weit abstehenden, spitzwinkligen, kaum abgestumpften Schulterecken. Das Basaldreieck ist schwach eingedrückt, der Höcker hinter ihm hoch und spitzig, die Profillinie vor und hinter ihm ziemlich tief konkav ausgerandet. Die Punktstreifen sind grob, ineinander undeutlich übergreifend; die alternierenden (primären) Zwischenräume, besonders der 2. und 4. sind höher, der 2. durch eine hohe Querleiste mit dem Höcker, dahinter durch eine viel niedrigere mit dem 4. Zwischenraum verbunden. Das Seitendach ist auf den Randästen sehr tief, narbig, auf den durchscheinenden Stellen, besonders innen viel feiner punktiert. Die Klauen sind innen ziemlich lang gekämmt, aussen undeutlich gerieft.

Laccoptera schultzei ist der *quadripunctata*, die über das ganze südliche China bis zur Spitze von Vorder Indien verbreitet ist, zunächst verwandt, unterscheidet sich aber durch die viel grössere Gestalt, höheren Höcker, tiefer ausgerandete Profillinie, nach hinten nicht verengten Halsschild mit gröber und länger rissiger Scheibe, seitlich weiter ausladende, spitzigere Schulter-

ecken, gröbere Punktstreifen mit höheren primären Zwischenräumen, gröber skulptiertes Seitendach.

Das einzige, der Beschreibung zugrunde liegende Stück wurde von Herrn W. Schultze gesammelt und ist in seiner Sammlung.

Auf den Philippinen sind bisher drei Arten *Laccoptera* aufgefunden worden:

a. Laccoptera tredecim-punctata F. Nur auf Palawan, wo sie von C. F. Baker bei Puerto Princesa gesammelt wurde.

b. Laccoptera philippinensis Blanch. Die letztere auf allen Inseln, besonders auf Luzon häufige Art wurde von Blanchard [4] beschrieben; eine genauere Beschreibung gab dann Boh. im III. Bande seiner Monographie 1855. Da Blanchard als Vaterland seiner Art, obwohl er sie *philippinensis* nannte, Sumatra angab, wo die hier in Betracht kommende Art nicht vorkommt, änderte ich den Namen in *luzonica;* [5] ich glaube jetzt aber, dass hiezu kein genügender Grund vorliegt, da Blanchard jedenfalls auch Stücke von den Philippinen vorlagen. *Laccoptera philippinensis* ist hinsichtlich der Punktierung der Flügeldecken und des Seitendaches sehr veränderlich; das reiche, mir in den letzten Jahren vorgelegene Material hat mich überzeugt, dass nicht nur die *L. 13-guttata* Wagen., deren Typus in meiner Sammlung ist, sondern auch die *manilensis* Wse., 1910, dieselbe Art sind. Die Synonymie ist daher:

LACCOPTERA PHILIPPINENSIS Blanch, 1853; Boh., 1855.
= *13-guttata* Wagen., 1881.
= *manilensis* Wse., 1910.
= *luzonica* nom. cat., 1914.
ab. *nigripennis* Wse., 1910.

c. Laccoptera schultzei sp. nov. Mindoro.

CASSIDA LUZONICA sp. nov.

Rotundata, nitida, rufotestacea, elytrorum disco nigromaculato et vittato; caput trapezoidale, planum, subpunctatum; prothorax subellipticus, antice minus quam postice rotundatus, longitudine vix duplo latior, disco vix punctulato; elytra latitudine parum longiora, basi leviter impressa, deinde subgibbosa et ruga parum elevata instructa, subregulariter punctatostriata; long. 6, lat. 5.25 mm.

LUZON, Ilocos Norte Province, Dungan-Dungan (W. Jark, mus. Hambg.); Burgos (coll. Schultze). Typus in mea collectione.

[4] Voy. Pôle Sud 4 (1853) 312.
[5] Junk-Schenkling, Coleop. Cat. pars 62 (1914) 82.

Rötlichgelb, mit wenig dunkleren Endgliedern der Fühler und schwarzer Zeichnung der Flügeldecken. Gewölbt, gerundet, mit der grössten Breite vor der Mitte der Flügeldecken, oben glänzend. Kopfschild trapezförmig, um mehr als die Hälfte länger als breit, mit flachem, glattem, glänzendem, lang dreieckigem Mittelfeld, das von feinen, konvergierenden Stirnlinien, die am Augenrande verlaufen, umsäumt wird. Die Fühler überragen wenig die Ecken des Halsschildes und haben 5 schwach verdickte und mattere Endglieder, die um die Hälfte länger als dick sind, das 3. Glied ist nur wenig länger als das 2.; die folgenden fast gleich lang.

Halsschild doppelt so breit als lang, elliptisch; hinten mehr als vorn gerundet; die abgerundeten Ecken liegen wenig vor der Längsmitte; die Oberseite ist sehr fein punktuliert. Die Flügeldecken sind an der Basis schwach ausgerandet, in den schräg, nicht ganz bis zur Mittellinie des Halsschildes vorgezogenen, spitzwinkligen, mässig scharfen Schulterecken fast ein Viertel breiter als der Halsschild, dahinter bis vor die Mitte sehr schwach erweitert, hinten breit verrundet; die Scheibe ist im Basaldreieck deutlich eingedrückt, dahinter undeutlich stumpf gehöckert; ihre Profillinie ist sehr stumpfwinklig gebrochen, hinten mit schwacher Neigung gerade abfallend; die Punktstreifen sind regelmässig, grob, aber nicht tief, ihre Zwischenräume innen breiter, aussen schmäler, stark glänzend, glatt, schwach gewölbt; die Höcker-Querleiste ist kräftig und gabelt sich am 2. Zwischenraum; sie ist gesättigter gelb als die übrige Scheibe und setzt sich in dieser Färbung auf dem hier breiteren zweiten Zwischenraum bis an die Basis fort; die schwarze Zeichnung besteht aus einer aussen von diesem Zwischenraum beginnenden Binde, die an der Basis quer über die Schulterbeule laufend, nach hinten biegt, aussen vom vorletzten Streif begrenzt wird, an der Seitendachbrücke bis zum 7. Streif tief ausgerandet ist und dann in Flecke aufgelöst, um die Scheibe hinten zur Naht zieht, wobei der letzte und hinten der vorletzte Zwischenraum, gelb bleiben; ausserdem sind der Höcker und einige Flecke innen, die eine aufgelöste Querbinde bilden, schwarz; die verbleibenden hellen Stellen sind sehr schwach überhöht; Seitendach breit, hinten nur wenig schmäler, glatt, mässig geneigt. Klauen ohne Zahn.

Cassida luzonica gehört zur Gruppe der *C. andrewesi* Wse., die in Südasien in zahlreichen Arten vertreten ist, welche zum Teil eine der *luzonica* ähnliche Zeichnung haben (*andrewesi, justa, gentilis* u. a.). Von den Philippinen ist aus dieser Gruppe

bisher nur eine Art, *C. abamita* m.[6] bekannt; diese ist der *luzonica* äusserst ähnlich, aber kleiner (5 mal 4.25 mm.), an den Seiten nicht erweitert, im Basaldreieck weniger tief eingedrückt, der Höcker schwächer, die Punktstreifen sind feiner, die schwarzen Zeichnungen auf eine hinten abgekürzte Längsbinde, die Höckerstelle und einen unscharfen Querwisch hinten reduziert; ferner ist der Kopfschild eingedrückt.

CASSIDA EXCÆCATA sp. nov.

Late subovata, nitida, rufotestacea, prothorace maculis duabus subeffusis brunneis, elytrorum disco nigro, maculis nonnuelis rugaque subelevatis laete testaceis; caput planum trapezoidale, sublaeve; prothorax ellipticus, lateribus late rotundatis, disco sublaevi; elytra latitudine vix dimidio latiora, angulis humeralibus subacutis, parum prominulis, disco regulariter punctatostriata, basi leviter retusa, subgibbosa; long. 5.5, lat. 4.75 mm.

LUZON, Trinidad (2 specimina in mea collectione a dom. Staudinger).

Breit-eiförmig, subtriangulär, glänzend, rötlichgelb, die Scheibe des Halsschildes mit zwei grossen unscharfen, durch einen schmalen, hellen Streif getrennten Flecken, die Flügeldecken schwarz mit rotgelben Flecken. Schildchen dunkel gerandet; die Brust in der Mitte bräunlich. Kopfschild um ein Viertel länger als breit, trapezförmig, mit glattem, flachem, dreieckigem Mittelfeld und dem Augenrande parallelen Stirnlinien. Die Fühler überragen wenig die Halsschildecken und haben 6 glänzende Basal- und 5 schwach behaarte, matte, verdickte Endglieder; das dritte Glied ist um die Hälfte länger als das zweite und auch merklich länger als das vierte; das fünfte und sechste sind noch etwas kürzer, so lang wie das zweite.

Halsschild elliptisch, um mehr als die Hälfte breiter als lang, an der Seite sehr breit gerundet und wenig kürzer als in der Mitte, mit glänzender, kaum merklich und sehr zerstreut punktulierter Scheibe. Flügeldecken an der Basis um ein Viertel breiter als der Halsschild, mit von diesem seitlich weit abstehenden und mässig vorgezogenen, spitzwinkligen, ziemlich scharfen Schulterecken; hinter diesen sind die Seiten zuerst noch ganz unmerklich erweitert und laufen dann schwach schräg zusammen; die Scheibe hat ziemlich grobe, fast regelmässige Punktstreifen, die auch hinten nicht feiner sind; die Zwischenräume sind kaum breiter; das Basaldreieck ist beider-

[6] Stett. Ent. Zeit. (1916) 349.

seits der Naht schwach eingedrückt, die Profillinie ist an der Höckerstelle, die nicht überhöht ist, winklig gebrochen, hinten anfangs gerade und horizontal. Der letzte Zwischenraum, eine tiefe Einbuchtung an der Seitendachbrücke, eine ebensolche hinten neben der Naht, zwei gemeinsame kleine Flecke, der eine hinter dem Schildchen, der andere hinter der Mitte, sowie je drei grosse, etwas reliefe Flecke hintereinander in der Mitte der Decke sind gelb; von diesen ist der erste rund an der Basis, der zweite gross und unförmlich auf der gegabelten Höcker-Querleiste, aussen ausgerandet, der dritte hinter der Mitte ist aus mehreren zusammengeflossen und bildet mit seinem Gegenüber und der Ausrandung an der Seitendachbrücke einen vor offenen Bogen. Klauen ohne Basalzahn.

Cassida excæcata ist der *luzonica* nahe verwandt, kleiner, schmäler. Die Schulterecken sind mehr zugespitzt und vorgezogen, die Flügeldecken viel mehr nach hinten verengt. Der Kopfschild ist kürzer, das dritte Flügelglied ist länger, die Höckerstelle weniger herausgehoben; die Zwischenräume sind schmäler; endlich ist die Zeichnung verschieden, da der Halsschild dunkle Flecken hat und auf der Scheibe der Flügeldecken nur einige stärker reliefe Flecke gelb bleiben.

THLASPIDOSOMA RIZALENSIS sp. nov.

Rotundata, nitida, glabra, subtus flava, antennis albidoflavis, prothorace elytrisque in discis laete testaceis, parum obscurius signatis, protecto elytrorum ramis duobus brunnescentibus, ceterum ut protectum prothoracis albidohyalino; prothorax longitudine vix duplo latior, angulis subobtusis pone medium; elytra basi emarginata, angulis humeralibus subacutis, basi leviter retusa, tum subgibbosa, subtiliter punctatostriata, punctis apice subeffusis; long. 10, lat. 9.5 mm.

Luzon, Mount Irid (*W. Schultze*).

Annähernd gerundet, mit der grössten Breite wenig vor der Mitte der Flügeldecken, kaum merklich länger als breit, wenig gewölbt, glänzend; unten lebhafter gelb, die Fühler ganz weissgelb, die Scheiben der Flügeldecken und des Halsschildes hell braungelb mit brauner, unscharfer Zeichnung, das Seitendach mit zwei mässig breiten, braunen Randästen, die bis an den Aussenrand reichen, sonst wie das ganze Vordach des Halsschildes glashell, weisslichgelb, durchscheinend. Die braunen Zeichnungen bilden auf der Halsschildscheibe zwei nach vorne konvergierende, kurze Längsbinden; auf den Flügeldecken sind ein kurzer Längsstreif innen von der Schulter und je eine Längsbinde,

braun, die im ersten Längsdrittel hinter dem rückwärtigen Ast der Höcker-Querleiste beginnt, anfangs den 3. und 4. Zwischenraum bedeckt und gegen die Naht konvergiert, wo sie nach dem zweiten Längsdrittel endet. Der vordere Randast des Seitendaches lässt einen glashellen Fleck aussen am Vorderrande frei. Fühler lang und sehr dünn; das 2. Glied kurz, nur doppelt so lang als dick, alle anderen drei bis viermal so lang als das zweite und vier bis fünfmal so lang als dick, unter sich wenig verschieden in der Länge. Kopfschild gut um die Hälfte länger als breit, schwach trapezförmig, mit seichter Mittelrinne und dichter Schagrinierung; die Oberlippe ist oben scharfkantig gerandet, bogenförmig, ober dem Munde weit und seicht ausgerandet, ohne Längskiel. Halsschild fast doppelt so breit als lang, annähernd elliptisch, aber vorne mehr als hinten gerundet, mit abgestumpften Ecken etwas nach der Mitte; Oberseite spiegelglatt. Flügeldecken an der Basis wenig breiter als der Halsschild, ziemlich tief ausgerandet, mit wenig über den Halsschild seitlich vortretenden, schwach vorgezogenen, spitzwinkligen, mässig scharfen Schulterecken; die Scheibe ist wenig gewölbt, sehr undeutlich gehöckert, mit fast geradliniger Profillinie hinter der Höckerstelle; von dieser geht eine flache, breite Querleiste aus, die sich gabelt und zwei schwach herausgewölbte Aeste bis zum 7. Punktstreif sendet, welche die Punktstreifen unterbrechen; auch gegen die Basis geht auf den ersten Zwischenräumen ein höher herausgewölbter, glatterer Ast; die Punktstreifen sind fein, an der Spitze fast erloschen, ihre Zwischenräume mehr als doppelt so breit. Das Seitendach ist fast so breit als eine Decke, glatt, flach ausgebreitet, der äusserste Randsaum schwach aufgebogen.

Die vorstehende Art ist von der zweiten, bisher von den Philippinen Inseln nachgewiesenen *Thlaspidosoma* sehr verschieden. Diese letztere *T. philippina* m.[6] hat auf der sonst gelben Oberseite einen schwarzen, hinten in einen kurzen Zapfen verlängerten Ring, der an der Höckerstelle einen rötlich-gelben Kreis umschliesst. Das Seitendach hat keinen Randast. Zur Zeit der Beschreibung war nur das Männchen bekannt, das sich durch die sehr langen Fühler, welche fast die Körperlänge erreichen, auszeichnet; seitdem liegt mir aus Surigao auf Mindanao auch ein von Baker gesammeltes Weibchen vor, dessen Fühler viel kürzer, nur von halber Körperlänge, sind; insbesonders ist das letzte Glied nur um die Hälfte, beim Männchen aber doppelt so lang als das zehnte.

[6] Stett. Ent. Zeit. 77 (1916) 350.

Die Arten der Gattung Thlaspidosoma.

1 (6). Höcker mässig hoch und spitz; die Profillinie hinter ihm nur seicht ausgerandet.
2 (3). Seitendach ohne Randast; Oberseite mit schwarzem Ring. Mindanao ..... *T. philippina* Sp.
3 (2). Seitendach mit Randästen, Oberseite ohne ringförmige Zeichnung.
4 (5). Seitendach mit zwei Randästen ..... *T. rizalensis* sp. nov.
5 (4). Seitendach nur hinten mit einem Randast, der wie die Scheiben des Halsschildes und der Flügeldecken pechschwarz ist; der Höcker mit der Querleiste, sowie eine aufgelöste Querbinde hinter der Mitte der Flügeldecken sind gelb (im Leben goldig), das Vor- und Seitendach sowie das Schildchen und ein unscharfer Fleck auf der Basalmakel des Halsschildes hellgelb. 8.5 mal 8 mm. Java ..... *T. horsfieldi* Boh.
6 (1). Der Höcker ist hoch, die Profillinie hinter ihm tief ausgerandet.
7 (10). Halsschild vorne weniger gerundet als hinten, so dass die Ecken vor der Mitte liegen.
8 (9). Die Punktstreifen der Flügeldecken haben grobe, dichter hintereinanderstehende Punkte; der Höcker ist niedriger, oben mehr abgestumpft, die Profillinie ist hinter ihm schwächer ausgerandet. Die dunklere Färbung der Oberseite ist nur ein helles Gelbbraun. Die Schulterecken sind weniger scharf..... *T. celebensis* sp. nov.[1]
9 (8). Die Punktstreifen haben sehr seichte, nicht dicht stehende Punkte und sind weniger regelmässig; der Höcker ist hoch, spitzig, die Profillinie ist hinter ihm tief ausgerandet. Die dunkle Zeichnung der Oberseite ist pechschwarz. Schulterecken durch eine Ausrandung der Seiten hinter ihnen scharf. Sumatra, Borneo. Bei der subsp. *limbata* Sp. fehlt der Randast hinter der Mitte des Seitendaches (Borneo) ..... *T. dohrni* Sp.

[1] *Thlaspidosoma celebensis* sp. nov. Breit gerundet, hell bräunlichgelb, die Scheiben und eine nicht bis an den Rand reichende, quere Makel hinter der Mitte des Seitendaches hell rötlichbraun; auf den Flügeldecken bleiben ein grosses, gemeinsames Dreieck, das bis über den Höcker reicht, und die Umgebung der Seitendachbrücke, heller. Die Fühler sind bis zum sechsten Gliede gelb (die übrigen Glieder fehlen); das vierte Glied ist doppelt so lang als das zweite und um die Hälfte länger als das dritte. Halsschild fast doppelt so breit als lang, vorne weniger als hinten gerundet, mit abgestumpften Ecken vor der Mitte. Flügeldecken mit vorgezogenen, spitzwinkeligen, aber nicht scharfen Schulterecken, mässig hohem Höcker, beiderseits, vorne tiefer, ausgerandeter Profillinie, groben, tiefen Punktstreifen und kaum breiteren, innen mehr gewölbten und höheren Zwischenräumen, die stellenweise durch kleine Querleisten verbunden sind. Seitendach breit und flach; die Seitendachbrücke geht nach innen nur bis zum achten Streif.

Von der ihr zunächst verwandten *T. dohrni* durch die oben angegebenen Merkmale, mehr elliptischem, vorne mehr gerundetem Halsschild, und der hinter den Schulterecken fehlenden Ausrandung verschieden; 9 mal 9 mm.

Celebes, Lakini Mont.

Das einzige von den Brüdern Sarasin gesammelte Stück ist im Museum in Basel.

10 (7). Halsschild vorne und hinten gleich gerundet; die mehr abgerundeten Ecken liegen in der Längsmitte.

11(12). Flügeldecken mit einer von einer Seitendachbrücke zur anderen laufenden, nach hinten gerichteten, rötlichgelben Querbinde; der vor dieser Binde gelegene Teil ist dunkel-pechbraun, der dahinter gelegene, ebenso wie der Randast hinter der Mitte des Seitendaches, und die Halsschild-Scheibe, heller holzbraun; 7.25 bis 7 mm; Assam, Mts. Patkoy..... *T. assamensis* Sp.[8]

12(11). Flügeldecken ohne rötlichgelbe Querbinde.

13(14). Die Scheiben des Halsschildes und der Flügeldecken sind schwarz mit gelben Relieffleckeu. Seitendach vorn mit einem abgekürzten, hinten mit einem bis an den Rand reichenden, schwarzen Ast. Das letzte Fühlerglied ist weissgelb, die Basalglieder haben helle Basis; das dritte Glied ist doppelt so lang als das zweite, kaum kürzer als das vierte; 9 mal 8 mm. Kinabalu auf Borneo ..... *T. gracilicornis* Sp.[9]

14(13). Scheiben des Halsschildes und der Flügeldecken, sowie ein Randast hinten hellbraun; auf den Flügeldecken sind hellgrüne Relieffleckе. Fühler gelbbraun. Flügeldecken hinten viel breiter abgestutzt 8.5 mal 8 mm. Sumatra.... *T. fallaciosa* Sp.

Genus METRIONA Weise

Ueber *Metriona circumdata* H. habe ich schon geschrieben[10] und zwei ihr nahe verwandte Arten aus Neu-Guinea und von den Molukken abgesondert; die *Metriona trivittata* F. wurde als Rasse der *circumdata* angesprochen; im übrigen habe ich schon damals darauf hingewiesen, dass die Art in eine Reihe von Lokalrassen zerfällt, deren Abgrenzung und Studium reicheres Material mit genauen Fundorten erfordern, als mir damals zur Verfügung stand.

Diese Voraussetzung ist seitdem teilweise erfüllt worden; insbesonders von den Philippinen liegen mir jetzt zahlreiche Stücke vor, die mir ermöglichen einige Lokalformen festzustellen.

METRIONA CIRCUMDATA H. subsp. MINDANAOENSIS subsp. nov.

Männchen sehr kurz und breit, Weibchen viel länger und schmäler. Halsschild vorne und hinten gleichartig gerundet, mit mässig schmal verrundeten Ecken in der Längsmitte; die Flügeldecken sind beim Männchen fast doppelt so breit, kaum länger als breit, mit seitlich vom Halsschild weit abstehenden, wenig scharfen schwach vorgezogenen Schulterecken; beim Weibchen sind die Flügeldecken nur um etwas mehr als die

[8] Vereinssch. d. Ges. Luxemb. Naturfr. (1926) 58.
[9] Stett. Ent. Ztg. 76 (1915) 288.
[10] Ann. Mus. Nat. Hung. 1 (1903) 128.

Hälfte breiter als der Halsschild, um ein Viertel länger als breit; die Schulterecken treten nach aussen weniger vor. Auf dem Halsschild ist vor dem Schildchen ein schmaler, an der Spitze kurz gegabelter Längsstrich; sehr selten setzt er sich vorne nach den Seiten fort und es zeigt sich an der Basis noch beiderseits ein schräger Querstrich, welche Zeichnung ganz klar zu der bei der typischen *circumdata* häufigeren Zeichnung mit zwei birnförmigen, gelben Flecken auf der Basalmakel hinüberleitet. Das Schildchen ist schwarz, oft mit einem gelben Flecke. Auf den Flügeldecken ist eine grosse schwarze Makel, welche an der Basis bis zum vorletzten, selten nur bis zum zweitletzten Punktstreif reicht, an der Seitendachbrücke fast immer tief bis zum sechsten Streif ausgerandet ist (diese Ausrandung fehlt bei einem einzigen Stücke von Nord-Mindanao), sich darauf wieder bis zum vorletzten Streif erweitert und hinten noch zweimal ausgerandet ist; auf jeder Decke sind normal sieben gelbe, kaum erhabene, aber von den Punktreihen nicht durchsetzte, daher glatte Flecke, einer an der Basis, ein grösserer neben der Höckerstelle, zwei dann hintereinander an der Naht, und drei in der Mitte der Decke, davon der mittlere mehr innen, der letzte schräg hinter der Ausrandung; oft fliessen einzelne derselben, besonders jener an der Höckerstelle, mit dem schräg hinter ihm stehenden zusammen, und die bekannte x-förmige Zeichnung wird angedeutet.

Metriona catenata, die eine entfernt ähnliche Zeichnung ist im allgemeinen, kleiner, mehr gewölbt, die Schulterecken springen seitlich weniger vor, die Flecke sind höher relief, die birnförmige Zeichnung des Halsschildes meist vollständig.

Metriona circumdata mindanaoensis ist auf Mindanao sehr häufig; ich kenne sie von Nord-Mindanao (ohne näheren Fundort), Dapitan, Dansalan, Zamboanga, Davao (*Baker, Warburg*); Surigao (*Schultze*), ferner von den Nachbar-Inseln Siargao: Cabuntug, und Samar: Catbalogan; bei diesem letzten Stücke ist der Halsschild einfärbig gelb; 5 mal 4.5 bis 5.5 mal 5 mm.

METRIONA CIRCUMDATA H. subsp. POLILLOENSIS subsp. nov.

Von dieser Form liegen mir nur zwei Stücke vor, die aber ganz übereinstimmen und von verschiedenen Sammlern auf der von Mittel-Luzon östlich gelegenen, kleinen Insel Polillo gesammelt wurden. Diese Form ist sehr gross (6 bis 5.25 mm), der

Halsschild ist vorne weniger als hinten gerundet, so dass die Ecken vor der Mitte liegen, die Flügeldecken haben kaum erweiterte Seiten und stark nach vorne, aber nicht nach aussen vorgezogene Schulterecken; sie sind auch verhältnismässig hoch gewölbt. Der Halsschild ist einfärbig gelb, auf den Flügeldecken sind die Naht bis zur Mitte, an der Höckerstelle breiter, und je eine Aussenbinde schwarz; letztere ist breit, aussen schwach ausgerandet, beginnt auf der Schulterbeule und geht bis hinter die Mitte schräg nach hinten, anfangs vom vorletzten, später vom drittletzten Streif aussen begrenzt.

Das eine der beiden Stücke ist in meiner Sammlung, das zweite in der von Herrn W. Schultze.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM EASTERN ASIA (DIPTERA), XIV [1]

By Charles P. Alexander
Of Amherst, Massachusetts

THREE PLATES

The majority of the species described herewith were taken by the Reverend David C. Graham on the border between western China and Tibet, the types being contained in the United States National Museum. A smaller number of Japanese crane flies were captured by Messrs. Esaki and co-workers at the Kiushiu Imperial University, and by Messrs. Imanishi, Machida, Nakamura, Sakaguchi, and Yasumatsu. The material sent by Messrs. Esaki and Yasumatsu is preserved in the entomological collection of the Kiushiu Imperial University; that sent by Messrs. Imanishi, Machida, Nakamura, and Sakaguchi is contained in my collection, through the kindly interest and coöperation of the entomologists in question. I wish to express my deep thanks to all of the above-mentioned gentlemen, and to Dr. John M. Aldrich, of the United States National Museum, through whose intervention I have been able to study the very valuable Graham material.

The crane flies from the China-Tibet border are of unusual interest in that they include certain specimens that were taken at the highest altitude ever reported for these flies; namely, 17,000 feet. The highest previous station was at Phusi-La, Tibet, altitude, 16,500 feet, recorded by Edwards.[2]

TIPULINÆ

TIPULA (SCHUMMELIA) ESAKIANA sp. nov. Plate 1, fig. 1; Plate 2, fig. 23.

Belongs to the *variicornis* group; antennæ (male) elongate, bicolorous; mesonotal præscutum light brown, with three darker brown stripes, the median one further divided by a capillary darker median vitta; fore femora dark brown, remaining femora

[1] Contribution from the entomological laboratory, Massachusetts State College.

[2] Ann. & Mag. Nat. Hist. X 1 (1928) 681–703.

more brownish yellow, with the tips narrowly dark brown; tibiæ and tarsi black; wings with a faint brown tinge, the outer portion of cell R_2 strongly infumed; m-cu connecting with M_{3+4} at about one-third its length; male hypopygium with the median lobe of the tergite relatively small, densely setiferous.

Male.—Length, about 9 millimeters; wing, 8.5; antenna, about 5.

Frontal prolongation of head testaceous-brown; nasus distinct; palpi with basal segments pale brown, the outer segments brownish black. Antennæ (male) elongate, as shown by the measurements; basal three segments yellow; succeeding segments bicolorous, the basal enlargement black, the elongate apical portion light yellow, on outer segments somewhat obscured. Head brown, with a narrow median darker vitta on vertex.

Mesonotal præscutum light brown, with three darker brown stripes, the median one paler at anterior end, divided by a capillary darker median vitta; scutal lobes light brown, each with two darker brown areas; scutellum and mediotergite darker brown, sparsely pruinose. Pleura obscure yellow, variegated with brown on anepisternum, ventral sternopleurite, dorsal pteropleurite, and on pleurotergite. Halteres yellow, the base of knob infuscated. Legs with the coxæ and trochanters yellowish testaceous; fore femora dark brown, paler at bases; middle and hind femora more obscure brownish yellow, the tips narrowly dark brown; tibiæ and tarsi black. Wings (Plate 1, fig. 1) with a faint brownish tinge, the outer three-fourths of cell R_2 strongly infumed; cell C pale brown, cell Sc yellow; very narrow brown seams along veins Cu, m-cu, and anterior cord; whitish areas before and beyond stigma and across cell 1st M_2; veins brown, white in the obliterative areas. Venation: m-cu connecting with M_{3+4} at about one-third its length.

Abdominal tergites obscure brownish yellow, narrowly margined laterally and caudally with blackish; basal sternites more yellowish, the outer ones darkened. Male hypopygium (Plate 2, fig. 23) with the ninth tergite, 9*t*, produced medially into a spatulate lobe that is narrower and smaller than in *insulicola*, the entire surface covered with conspicuous erect setulæ, not transversely furrowed and nearly glabrous, as in *insulicola*. Inner dististyle, *id*, with the beak not in alignment with the margin of the blade, but with a slight but distinct emargination at the point of union of the two.

Habitat.—Japan (Kiushiu).

Holotype, male, Sobosan, Bungo, July 13, 1931 (*Esaki and Fujino*).

Tipula (*Schummelia*) *esakiana* is named in honor of my dear friend and colleague, Professor Teiso Esaki, to whom I am particularly indebted for coöperation in making known the rich tipulid fauna of Japan. In its small size and general appearance, it much resembles *T.* (*S.*) *acifera* Alexander (Japan: Shikoku), which, however, has the male hypopygium entirely different in structure, the median lobe of the tergite being nearly cylindrical or slightly narrowed outwardly.

TIPULA (SCHUMMELIA) HONORIFICA sp. nov. Plate 1, fig. 2; Plate 2, fig. 24.

Belongs to the *continuata* group; mesonotal præscutum obscure yellow, with three conspicuous brown stripes that are narrowly bordered by still darker brown, the midline of præscutum thus with a double line of the latter color; antennal flagellum black; head orange, anterior vertex with a capillary black vitta; pleura variegated dark brown and obscure yellow; femora obscure yellow, with a nearly terminal dark brown ring; wings cream-yellow, the costal border brighter yellow, the disk with a heavy dark pattern; Rs about one-half longer than m-cu; male hypopygium with the inner dististyle extended into an acute blackened beak.

Male.—Length, about 13 millimeters; wing, 15.

Frontal prolongation of head orange, brown on sides; nasus lacking; palpi brown, the first segment paler. Antennæ with scape and pedicel yellow; flagellum black; flagellar segments with basal enlargement small, verticils unilaterally arranged. Head deep orange, the anterior vertex with a capillary black median line.

Anterior pronotum brown, narrowly yellow on median line; posterior pronotum broadly sulphur-yellow medially. Mesonotal præscutum obscure yellow, with three, conspicuous, dark brown stripes that are narrowly bordered by still darker brown, the mesal borders of the intermediate stripes forming two delicate parallel vittæ that lie so close as to appear almost single; scutal lobes dark brown, the medial region paler; scutellum and mediotergite brown, obscurely paler on median portion. Pleura chiefly dark brown, variegated by obscure yellow on dorsal anepisternum and sternopleurite; meron chiefly yellow; ventral pleurotergite conspicuously light yellow. Halteres elongate, the stem dirty yellow, the knobs clear light yellow. Legs with the coxæ brownish yellow; trochanters obscure yellow; femora ob-

scure yellow, near tips narrowly dark brown, the extreme tips pale; tibiæ and tarsi black, the extreme bases of former somewhat paler. Wings (Plate 1, fig. 2) with the ground color cream-yellow, cells C and Sc brighter yellow; stigma dark brown; an unusually heavy, paler brown, clouded pattern in most cells, restricting the ground to include most of cell M_1, small areas before and beyond stigma, a small spot at origin of Rs, across fork of M and with areas in cells M, Cu, 1st A, and 2d A; cord, outer medial veins, and vein 2d A narrowly seamed with still darker brown; the brown clouds in cell M with triangular, more grayish centers. Venation: Rs relatively long for this subgenus, about one-half longer than m-cu; R_{1+2} entire, the distal half pale, without trichia.

Abdomen with the basal segments obscure yellow, variegated laterally with brown; segments six to nine black. Male hypopygium (Plate 2, fig. 24) with the caudal margin of tergite, 9*t*, deeply notched medially, the lateral lobes broadly rounded; notch relatively narrow, the margin heavily blackened; viewed from the side, the lateral lobes project ventrad into a small acute tooth; viewed from beneath and caudally, the whole outer margin of the tergite on ventral side is produced as an intensely blackened flange, 9*t*. Outer dististyle, *od*, long, cylindrical, with conspicuous setæ. Inner dististyle, *id*, small and simple, roughly triangular in outline, extended into an acute blackened spine, the entire cephalic border similarly blackened. Eighth sternite, 8*s*, with dense, long, yellow setæ on either side of a narrow, nearly glabrous, median area.

Habitat.—China-Tibet border.

Holotype, male, Yin-Kuan-Tsai, altitude 13,000 to 15,000 feet, July 25, 1930 (*Graham*).

The nearest regional ally of the present fly is the smaller *Tipula* (*Schummelia*) *chumbiensis* Edwards (Tibet), which differs in the short Rs, weakly patterned wings, differently colored femora, and details of structure of the male hypopygium.

TIPULA (ACUTIPULA) INCORRUPTA sp. nov. Plate 2, fig. 25.

General coloration of thorax yellowish gray, the præscutum with four brown stripes; antennæ with basal three segments yellow, the succeeding segments weakly bicolorous, the basal enlargement dark brown, the remainder paler brown, the outer segments more uniformly darkened; wings pale brown, with an oblique whitish band before cord; cells beyond cord not variegated with pale areas; a dark spot in cell Cu before mid-

length; abdomen with basal four segments yellowish, the outer segments blackened; apical lobe of tergite simple, spiculose; eighth sternite unarmed.

Male.—Length, 15 to 16 millimeters; wing, 18 to 19.

Frontal prolongation of head light yellow dorsally, the sides broadly infuscated; nasus conspicuous, yellow; palpi dark brown. Antennæ (male) with the basal three segments yellow, the succeeding segments weakly bicolorous, brown, the basal enlargements dark brown; outer segments more uniformly dark brown; terminal segment reduced to a mere thimble; verticils a little shorter than the segments. Head yellowish gray.

Pronotum obscure yellow medially, darker laterally. Mesonotal præscutum yellowish gray, with four brown stripes, the intermediate pair widely separated; posterior sclerites of mesonotum chiefly yellowish gray. Pleura yellowish gray. Legs with the coxæ yellowish gray; trochanters yellow, femora chiefly brown, the bases restrictedly light yellow, the tips narrowly still darker brown; tibiæ and tarsi rather light brown. Wings with a nearly uniform pale brown tinge; prearcular and costal regions light yellow; an oblique whitish band before cord, extending into base of cell M_3; a less distinct pale spot in outer third of cell M, adjoining vein Cu; cell Cu pale, with a dark spot before midlength; cells beyond cord uniformly darkened; veins brown, paler in the obliterative areas. Venation: Petiole of cell M_1 longer than m.

Abdomen with basal four segments orange-yellow, the tergites narrowly darkened sublaterally; segments five to nine, including hypopygium, black, the outer dististyle of the latter conspicuously whitened. Male hypopygium (Plate 2, fig. 25) with the apical lobe of the ninth tergite, 9*t*, simple, spiculose. Inner dististyle, *id*, as shown, the spinulose setæ at apex relatively sparse. Eighth sternite, 8*s*, with the caudal margin rounded, not at all produced, the median region with a roughly triangular area of setæ.

Habitat.—China-Tibet border.

Holotype, male, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930 (*Graham*). Paratopotypes, 3 males. Paratype, 1 male, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930 (*Graham*).

The present fly rather closely resembles *Tipula* (*Acutipula*) *yunnanica* Edwards in coloration, differing in the details of pattern of antennæ, thoracic notum, and wings, and in the structure of the male hypopygium, notably in the conformation of the

eighth sternite. There are evidently several other allied species of this particular group of flies in the high mountains of central Asia.

The subgenus *Acutipula* Alexander, as modified by Edwards, is represented by numerous species in the eastern Palæarctic region. Among these may be listed from the higher Himalayas, western China, and Tibet, the following species: *Tipula atuntzensis* Edwards, *biramosa* sp. nov., *brunnirostris* Edwards, *cinctoterminalis* Brunetti, *fumicosta* Brunetti, *fumifascipennis* Brunetti, *graphiptera* sp. nov., *incorrupta* sp. nov., *intacta* Alexander, *interrupta* Brunetti, *megaleuca* sp. nov., *munda* Brunetti (*vicaria* Walker, preoccupied), *princeps* Brunetti (including *fuscinervis* Brunetti), *quadrinotata* Brunetti, *robusta* Brunetti, *tenuipes* Brunetti (includes *walkeri* Brunetti, a re-naming of *fulvipennis* Walker, nec De Geer), *subturbida* Alexander, and *yunnanica* Edwards. *Tipula brunnicosta* Brunetti is more doubtfully a member of the subgenus. Additional species from eastern Siberia, Japan, Formosa, and Malaysia, include *bipenicillata* Alexander, *bubo* Alexander, *cockerelliana* Alexander, *de meijerei* Edwards, *kuzuensis* Alexander, *saitamae* Alexander, *shirakii* Edwards, *tokionis* Alexander, and *turbida* Alexander.

TIPULA (ACUTIPULA) GRAPHIPTERA sp. nov.

Allied to *bubo;* general coloration gray, the præscutum with four darker gray stripes that are narrowly bordered by darker; antennæ bicolorous; femora yellow, the tips conspicuously blackened; wings brown, variegated with white, forming longitudinal areas that are narrowly connected across cell 1st M_2; abdominal tergites reddish brown, darker sublaterally and with a capillary median vitta.

Female.—Length, about 25 millimeters; wing, 21 to 22.

Frontal prolongation of head dark reddish brown, the dorsomedian portion and nasus dark brown; palpi black. Antennæ with the scape and pedicel obscure yellow; flagellum obscurely bicolorous, the basal enlargement of each segment black, the remainder brownish yellow, the bicolorous effect persisting to the end of organ; verticils long and conspicuous. Head gray, the posterior vertex with vague indications of a capillary darker line.

Pronotum ocherous. Mesonotal præscutum yellowish gray, with four darker gray stripes that are vaguely and insensibly bordered by brown, most evident along the mesal edge of the

lateral stripe; scutum light gray, each lobe with a darker gray area that is bordered by brown; median line of scutum with a brown area; scutellum and mediotergite gray. Pleura gray, somewhat darker on the anepisternum and ventral sternopleurite; ventral pleurotergite more yellowish; dorsopleural membrane ocherous. Halteres with stem reddish brown, the knobs darker. Legs with the coxæ gray pruinose; trochanters yellow; femora yellow, the tips conspicuously blackened; tibiæ obscure yellow, the tips narrowly dark brown; tarsal segments passing into brown. Wings brown, conspicuously variegated by white areas, forming narrow longitudinal streaks, as follows: Bases of cells Cu, 1st A, and 2d A, the apices of these cells extensively darkened; a square brown spot before midlength of cell Cu; a large whitish area before cord, occupying cell R_1, outer half of R, and at near midlength of M; a narrow outer white stripe includes the narrow bases of cells M_1 to M_4, inclusive, together with the outer half of cell R_5, the two white fasciæ last described being connected across basal half of cell 1st M_2; veins brown, somewhat paler in the white areas; prearcular region brownish yellow.

Abdominal tergites reddish brown, with a very narrow but almost continuous capillary, brown, median vitta, and much broader sublateral stripes that are brown internally and more reddish brown externally; lateral borders of tergites buffy; sternites chiefly yellow. Ovipositor with the cerci long and straight.

Habitat.—China-Tibet border.

Holotype, female, Yu-Long-Si, altitude 15,600 feet, July 23, 1930 (*Graham*). Paratypes, 1 female, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930; 1 female, Tatsienlu, altitude 8,000 to 9,000 feet, August 16, 1930.

Tipula (*Acutipula*) *graphiptera* is most similar to *T.* (*A.*) *bubo* Alexander, differing most evidently in the distribution of the white wing areas, the ones in cells R and M being broadly connected, whereas in *bubo* they are broadly interrupted and separated across the outer ends of cells R and M.

TIPULA (ACUTIPULA) MEGALEUCA sp. nov.

Allied to *bubo;* general coloration gray, the præscutum with four darker gray stripes; antennal flagellum bicolorous; legs yellow; wings brown, with an unusually wide, white, longitudinal stripe extending the entire length; abdominal tergites buffy, with a broad blackish sublateral stripe on either side and a narrow, broken, median brown vitta.

Female.—Length, about 25 millimeters; wing, 23.

Frontal prolongation of head brown, sparsely pruinose; nasus elongate; palpi brownish black. Antennæ with scape and pedicel yellow; flagellum bicolorous, the basal enlargement of each segment brown, the remainder of the segment obscure yellow, on outer segments passing into brownish yellow; verticils much longer than the segments. Head gray.

Mesonotal præscutum light gray, with four darker gray stripes that are relatively ill-delimited against this ground; scutum light gray, the lobes variegated with darker gray; posterior sclerites of mesonotum gray, the caudal portion of mediotergite more yellowish. Pleura chiefly ocherous or yellow, the anepisternum and ventral sternopleurite more grayish; ventral pleurotergite immediately above root of halteres conspicuously infuscated. Halteres with the extreme base of stem and apex of knob pale, the intermediate portion dark brown. Legs with the coxæ and trochanters obscure yellow; remainder of legs pale yellow, only the outer tarsal segments darkened. Wings with the ground color brown, extensively striped longitudinally with white; prearcular region light yellow; the white longitudinal stripe includes the base of cell 2d A, all but the outer ends of cells 1st A and Cu, crosses into cell M at beyond midlength, thence to wing tip, involving the outer third of cell R, much of cell R_1 before stigma, all of 1st M_2, broad bases of cells M_1, 2d M_2, M_3, and M_4, and all but the basal-cephalic portion of cell R_5; a small brown spot before midlength of cell Cu represents the area commonly found in this subgenus; veins brown, paler in the whitish areas. Venation: R_{1+2} persistent; m-cu longer than Rs; M longer than petiole of cell M_1.

Abdominal tergites buffy yellow, with a broad, blackish, sublateral stripe on either side; dorsomedian area of tergite with a very narrow and interrupted median brown vitta, broken on posterior fourth of each segment; lateral borders of tergite broadly and conspicuously pale; basal tergite dark brown; basal sternites chiefly pale, the outer segments more darkened. Ovipositor with the cerci long and straight, slender; hypovalvæ shorter and a little deeper.

Habitat.—China-Tibet border.

Holotype, female, Tatsienlu, altitude 8,000 to 9,000 feet, August 16, 1930 (*Graham*). Paratopotype, female.

Tipula (*Acutipula*) *megaleuca* is quite distinct from all other species that are allied to *bubo* Alexander, in the yellow legs and very conspicuous, uninterrupted, white wing stripe.

TIPULA (ACUTIPULA) BIRAMOSA sp. nov. Plate 1, fig. 3; Plate 2, figs. 26, 27.

Allied to *munda;* mesonotum gray, the pronotum and pleura yellow; wings unmarked except for the stigma and brown costal border; male hypopygium with the inner dististyle produced caudally into two slender spines.

Male.—Length, about 22 to 24 millimeters; wings, 24 to 25.5.

Frontal prolongation of head deep orange; nasus long and slender; palpi dark brown, the incisures of the intermediate segments pale. Antennæ relatively short, slightly shorter than the palpi; basal three segments yellow, succeeding segments bicolorous, the basal enlargement black, the apical portion yellow; terminal segment small, dark-colored, subequal to the basal enlargements of the other segments; verticils very long and conspicuous, as in the group. Head gray.

Pronotum obscure orange-yellow. Mesonotum gray, the præscutum without distinct stripes. Pleura obscure yellow, the dorsopleural membrane more orange. Halteres brown. Legs with the coxæ and trochanters light yellow; femora light brown, the tips dark brown or black; tibiæ and basal three tarsal segments light brown, the tips narrowly and insensibly darkened; terminal tarsal segments black. Wings (Plate 1, fig. 3) grayish subhyaline, the stigma and costal region more infumed, especially the brown cell Sc; central portion of disk in cells M and Cu somewhat clearer, more grayish subhyaline; obliterative areas at cord restricted in area and inconspicuous; veins brown. Venation: Second section of vein M_{1+2} gently arcuated, widening cell 1st M_2; petiole of cell M_1 subequal to or a little shorter than m.

Basal abdominal tergites yellow, with a nearly lateral, brown, longitudinal stripe that widens on the fourth and succeeding segments to cover almost the whole sclerite, the lateral margins pale gray; basal four or five sternites yellow, the outer segments and hypopygium dark brown. Male hypopygium (Plate 2, fig. 26) with the tergite, 9*t*, chiefly fused with the sternite, 9*s;* basistyle fused with sternite, except beneath. Ninth tergite, 9*t*, tumid and dark-colored, with numerous setæ, the caudal margin produced medially into a slender yellow rod that is weakly bifid at apex, each half set with conspicuous black spicules. Outer dististyle (Plate 2, fig. 27, *od*) broadly obtuse, pale, with unusually sparse setæ that are chiefly marginal in distribution. Inner dististyle, *id*, with the caudal margin produced into two slender sclerotized horns. Eighth sternite,

8*s*, with a rounded median notch, on either side of which is a dense brush of long yellow setæ.

Habitat.—China (Szechwan).

Holotype, male, Fu-Lin, altitude 3,800 to 8,200 feet, 1928 (*Graham*). Paratopotype, male.

The nearest described ally is *Tipula* (*Acutipula*) *munda* Brunetti, which differs conspicuously in the longer, more nearly unicolorous antennæ and in the structure of the male hypopygium, notably of the inner dististyle.

TIPULA (VESTIPLEX) GRAHAMI sp. nov. Plate 1, fig. 4; Plate 2, fig. 28.

Size large (wing, over 20 millimeters); mesonotum gray, the præscutum with four more olive-gray stripes that are narrowly bordered by brown; tips of femora narrowly black; wings brown, variegated by darker brown and cream-colored areas, including a conspicuous postarcular brown area in cells R and M; male hypopygium with the tergite divided medially by pale membrane; basistyle produced dorsally into a powerful black spine.

Male.—Length, about 19 to 20 millimeters; wing, 22 to 23.

Female.—Length, about 27 to 30 millimeters; wing, 23 to 25.

Frontal prolongation of head narrowly yellow above, somewhat darker on sides; palpi dark brown. Antennæ (male) 12-segmented, relatively short, about as long as the palpus; scape and pedicel yellow; basal segment of flagellum brown, the outer segments uniformly darker brown; basal enlargements of segments weak; verticils exceeding the segments; terminal segment small. Front light yellow, posterior sclerites more brownish yellow.

Mesonotal præscutum gray, with four more olive-gray stripes that are narrowly bordered by brown; interspaces more brownish gray; scutum yellowish gray, the lobes with olive-gray areas, the median region with a capillary dark line; scutellum yellow, with a median brown vitta; mediotergite reddish brown, with a capillary brown central line. Pleura grayish yellow, the dorsopleural membrane yellow. Halteres yellow, the knobs dark brown, their apices pale. Legs with the coxæ yellowish gray; trochanters yellow; femora pale brown, the tips narrowly black, the amount about equal on all legs; tibiæ and tarsi brown. Wings (Plate 1, fig. 4) with the ground color brown, variegated by darker brown and cream-colored areas; the darkest area is the stigma; paler brown spots in bases of cells R and M, at origin of Rs, in cell R_1 just before the stigma, and along

anterior cord; the creamy areas include a poststigmal fascia extending from C to cell R_5 but not connecting with the more whitish obliterative streak across cell 1st M_2; other large creamy areas in cell R_1 beyond origin of Rs, subbasally in cells R and M, a large area before outer end of cell M, most of cell Cu and as conspicuous markings at base and near outer end of the anal cells; veins brown. Venation: Petiole of cell M_1 short, less than m, usually about one-half the length; m-cu on M_4 a short distance beyond origin.

Basal four abdominal segments orange-yellow, the tergites narrowly trivittate with dark brown, the lateral areas broadly interrupted; remaining abdominal segments, including hypopygium, black. Male hypopygium (Plate 2, fig. 28) with the suture between tergite, 9*t*, and sternite, 9*s*, poorly indicated; basistyle entirely cut off by a suture. Ninth tergite, 9*t*, completely divided on midline by pale membrane, each half with an outer triangular brown lobe and a conspicuous blackened lobe nearer the midline; mesal region of cephalic portions of tergite densely setiferous. Basistyle, *b*, produced dorsad and slightly caudad into a stout black spine. Outer dististyle, *od*, dusky, clavate in outline, with abundant small setæ. Inner dististyle, *id*, as shown. Eighth sternite, 8*s*, with the median region broadly pale and membranous, giving the erroneous impression of being marginate on caudal border.

Habitat.—China-Tibet border.

Holotype, male, Tatsienlu, altitude 8,000 to 9,000 feet, August 16, 1930 (*Graham*). Allotopotype, female. Paratopotypes, 1 female; paratypes, 1 male, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930; 5 females, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930.

Tipula (*Vestiplex*) *grahami* is named in honor of the collector, the Reverend David C. Graham, who has added very materially to our hitherto scanty knowledge of this prolific region. The species is readily told from other regional members of the subgenus by the combination of large size, darkened area near arculus of wings, and the structure of the male hypopygium, as the divided ninth tergite and produced basistyle. The latter feature is found in several other smaller members of the group, as *himalayensis*, *nigroapicalis*, *pleuracantha*, *serricauda*, *serridens*, *styligera*, *subtincta*, and probably others.

The subgenus *Vestiplex* Bezzi, as redefined by Edwards to include the *arctica* and *himalayensis* groups of the genus, is

well-defined in the female sex by the somewhat remarkable ovipositor, with powerful serrulate cerci and greatly reduced hypovalvæ. The group is greatly developed in the eastern Palæarctic region. Among the species known from the higher Himalayas, western China and eastern Tibet, are the following: *avicularia* Edwards, *divisotergata* Alexander, *edentata* Alexander, *grahami* sp. nov., *himalayensis* Brunetti, *inaequidentata* Alexander, *nigroapicalis* Brunetti, *nigrotibialis* Brunetti, *pleuracantha* Edwards, *quasimarmoratipennis* Brunetti, *reposita* Walker (including *brevis* Brunetti), *scandens* Edwards, *styligera* Alexander, *subscripta* Edwards, *subtincta* Brunetti and *tardigrada* Edwards. Additional species from eastern Siberia, eastern China, Japan, and Formosa include *asio* Alexander, *biserra* Edwards, *kuwayamai* Alexander, *nokonis* Alexander, *serricauda* Alexander, *serridens* Alexander, *subapterogyne* Alexander, *subcentralis* Alexander, *teshionis* Alexander, and *verecunda* Alexander. Species with marbled wings that closely simulate members of this subgenus but do not belong to *Vestiplex* include *bodpa* Edwards, *marmoratipennis* Brunetti, *tesselatipennis* Brunetti, *trilobata* Edwards, and members of the *thibetana* group, as *gregoryi* Edwards, *griseipennis* Brunetti, *hobsoni* Edwards, *thibetana* de Meijere, *waltoni* Edwards, and *wardi* Edwards.

TIPULA (LUNATIPULA) TRANSFIXA sp. nov. Plate 1, fig. 5; Plate 2, fig. 29.

General coloration of thorax buffy to gray, the præscutum with four ill-defined brown stripes; antennæ (male) elongate, if bent backward extending to the second abdominal segment, chiefly yellow; femora and tibiæ yellow, the tips narrowly brownish black; wings with a strong yellow tinge, the stigma darker; small cream-colored areas before and beyond stigma, in cell 1st M_2 and in outer end of cell M; abdomen yellow, the subterminal segment more or less darkened; male hypopygium with a pair of pale spatulate blades and another pair of decurved hooks jutting caudad from the notch of the ninth sternite.

Male.—Length, 14 to 17 millimeters; wing, 15 to 17.

Female.—Length, 17 to 22 millimeters; wing, 14 to 17.

Frontal prolongation of head yellowish; nasus long and slender; palpi brownish yellow to brown. Antennæ (male) relatively long, if bent backward extending about to the base of the second abdominal segment; in female shorter, about reaching the wing root; organ chiefly yellow, the basal enlargements of the segments small and darkened: verticils shorter than the seg-

ments (male) or a little longer than segments (female). Head dull gray, more yellowish gray in front.

Mesonotal præscutum buffy, more grayish laterally, with four brown stripes that are relatively ill-defined; scutal lobes gray, variegated with brown; posterior sclerites of mesonotum buffy with vague indications of a capillary brown line. Pleura buffy gray. Halteres yellow, the knobs infuscated. Legs with the coxæ buffy gray; trochanters yellow; femora and tibiæ yellow, the tips narrowly brownish black, somewhat broader on the former; basitarsi brownish yellow, the tips and remainder of tarsi dark brown; tibial spur formula 1–2–2; claws very small, simple. Wings (Plate 1, fig. 5) with a strong yellow to weakly brownish yellow tinge, the costal region more saturated; stigma brown; more cream-yellow areas before and beyond the stigma, across cord in cells M and 1st M_2, and as a small spot in cell M adjoining vein Cu; veins brown. Squama with a few small setæ. Venation: R_{1+2} entire; M_{3+4} about one-half the basal section of M_3; m usually longer than the petiole of cell M_1.

Abdomen yellow, in cases with segment eight (male) slightly darkened. Male hypopygium (Plate 2, fig. 29) of moderate size; tergite, 9*t*, and basistyle, *b*, cut off by sutures. Ninth tergite, 9*t*, transverse, the caudal margin with a broad U-shaped notch that embraces the whole caudal end of the sclerite, the median portion with a further small notch; caudal margins of tergite blackened and microscopically roughened near midline. Outer dististyle small, narrowly clavate in outline. Inner dististyle *id*, with two blackened apical points, the outer more slender. From the notch of the ninth sternite jut two pairs of appendages, 9*s*, consisting of an upper pair of pale spatulate blades and a more ventral pair of decurved hooks; a pendant lobe at caudal-mesal region of ninth sternite. Eighth sternite, 8*s*, moderately sheathing, its caudal margin straight, without armature of lobes or enlarged setæ. Ovipositor with smooth, relatively slender valves; hypovalvæ small.

Habitat.—China-Tibet border.

Holotype, male, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930 (*Graham*). Allotype, female, Tatsienlu, altitude 8,000 to 9,000 feet, August 16, 1930. Paratopotypes, 4 of both sexes; paratypes, 9 of both sexes, with allotype; 1 male, Jedo Pass, altitude 12,000 to 15,000 feet, July 17 and 18, 1930; 1 female, Yu-Long-Si, altitude 15,600 feet, July 28, 1930; 1 male, near Tang-Gu, 14,000 feet, August 3 to 6, 1930 (*Graham*).

There are rather numerous, generally similar species of *Lunatipula* in Europe and eastern North America but I know of no regional forms that are at all similar to the present fly. The relatively elongate antennæ, the deeply saturated wings, with restricted paler pattern, and, especially, the structure of the male hypopygium, serve to define the present fly. It may be noted that the peculiar armature jutting from the notch of the ninth sternite, which does not seem to represent any phallosomic development, is often withdrawn into the body so as to be invisible in dry specimens.

TIPULA OREADA sp. nov. Plate 1, fig. 6; Plate 2, fig. 30.

Size large (wing, male, 24 millimeters); general coloration gray, the præscutum with a median brown stripe that is narrowly margined laterally with dark brown; lateral stripes less distinct; wings yellow, the veins narrowly seamed with dark brown, broader along cord; vein R_3 sinuous, slightly narrowing the cell on basal half; male hypopygium with the basistyle produced caudad into a long obtuse point; a single dististyle.

Male.—Length, about 20 millimeters; wing, 24.

Frontal prolongation of head of moderate length, brownish gray; nasus short and stumpy; palpi with basal segment dark brown, the remainder black. Antennæ with the scape and pedicel obscure orange; flagellum brownish black; basal enlargements of segments moderately developed, with long conspicuous verticils that are about equal in length to the segments. Head yellowish gray, the posterior vertex more infuscated laterally.

Pronotum brown laterally, more obscure yellow medially. Mesonotal præscutum light gray, with three stripes, the lateral stripes darker gray and ill-defined; median stripe more brownish gray, clearly defined by narrow dark brown borders that are broader on cephalic half of stripe; posterior sclerites of mesonotum light gray, the scutal lobes marked with vague darker gray areas; posterior portions of scutellum and mediotergite more dusky. Pleura gray, the ventral pleurotergite light yellow. Halteres dusky, the apices of both stem and knob pale. Legs with the coxæ gray; trochanters obscure yellow; femora and tibiæ dark reddish brown, the outer tarsal segments blackened; tibial spur formula 1–?–2, middle legs broken; claws small, with basal spine. Wings (Plate 1, fig. 6) relatively broad; ground color light yellow, the prearcular region deeper yellow; a much restricted dark brown pattern at origin of Rs and along cord; all longitudinal veins similarly seamed very nar-

rowly with dark brown; very pale gray clouds in centers of most cells, restricting the ground to an oblique band at cord, a post-stigmal area in cells R_2 and R_3, bases of cells R and M, outer ends and bases of cells Cu and 1st A, and most of cell 2d A; veins dark. Squama setiferous. Venation: R_{1+2} entire; R_3 sinuous, slightly narrowing cell R_3 before midlength; petiole of cell M_1 shorter than m; m-cu at fork of M_{3+4}.

Basal abdominal tergites obscure yellow, trivittate with dark brown, the lateral stripes more pruinose and interrupted on caudal portions of segments; lateral margins of tergites on outer two-thirds more grayish; outer segments and hypopygium chiefly blackened. Male hypopygium (Plate 2, fig. 30) very large; suture between tergite, 9*t*, and sternite, 9*s*, complete. Ninth tergite, 9*t*, large, the caudal margin with a very conspicuous U-shaped notch, at base of which lie two small triangular points; lateral lobes flattened, their tips obliquely truncated. Basistyle, *b*, cut off by a complete suture, its outer end produced caudad into a long subcylindrical lobe that narrows gradually to the blunt tip, the dorsal surface of this lobe with scattered, long, pale setæ. Dististyle, *d*, single, the outer margin at base with a setiferous lobe which presumably is the homologue of the outer dististyle in those species where this is more evidently cut off from the main body of style. Phallosome, *p*, a small subquadrate mass. Eighth sternite, 8*s*, deeply notched medially, with an additional setiferous lobe on either side, together with a dusky liguliform lobe in the notch.

Habitat.—China-Tibet border.

Holotype, male, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930 (*Graham*).

Tipula oreada may perhaps be referred to the subgenus *Nippotipula* Matsumura but differs from the general type of the group in certain regards. The reduced wing pattern and the peculiar male genitalia, especially the long-produced basistyles, should make the species readily distinguishable among the large regional species allied to *pulcherrima* Brunetti and *thibetana* de Meijere.

TIPULA VARIIPETIOLARIS sp. nov. Plate 1, fig. 7; Plate 3, fig. 31.

General coloration gray; præscutum with three darker gray stripes; halteres dark brown; legs reddish brown, the tips of femora and tibiæ narrowly blackened; wings brown, variegated with white including a central streak that extends to the apex in cell R_5; petiole of cell M_1 variable in length, from subequal to

m to quite lacking; male hypopygium with the outer dististyle very small.

Male.—Length, 16 to 17 millimeters; wing, 19.

Frontal prolongation of head stout, gray pruinose; nasus lacking; palpi black, the terminal segment subequal to the second and third taken together. Antennæ (male) of moderate length, if bent backward extending nearly to wing root; scape and pedicel obscure orange; flagellum black; segments with rather conspicuous basal enlargements; verticils subequal to or a little shorter than the segments. Head gray, suffused with brown, the occiput and narrow orbits clearer gray.

Mesonotal præscutum light gray, with three darker gray stripes, the median stripe narrowly margined with brown, the narrow lateral stripes more uniformly darkened; lateral borders of præscutum more or less darkened; posterior sclerites of mesonotum clearer gray, the midline of mediotergite impressed on posterior half. Pleura gray; dorsopleural membrane yellowish brown. Halteres dark brown. Legs relatively stout; coxæ pruinose; trochanters brownish yellow; femora and tibiæ reddish brown, the tips very narrowly blackened, especially of the tibiæ; tarsi black, the proximal ends of basitarsi paler; tibial spur formula 1–2–2, the spurs long and slender, setuliferous; claws small, simple. Wings (Plate 1, fig. 7) with a strong brown suffusion in cells beyond cord, the basal cells paler; prearcular and costal regions more yellowish; a whitish streak runs almost the length of the wing, following vein M to cord, thence through cells 1st M_2 and bases of M_1, 2d M_2, and M_3 to the apex in distal half of R_5; other white streaks along vein M_4, outer third of 1st A, and in basal half of cell 1st A; the pale areas in stigmal region much reduced or lacking, most evident as a weak poststigmal brightening; veins brown. Squama naked; macrotrichia of veins beyond cord short but relatively numerous, lacking on distal thirds of veins R_{1+2} and R_3. Venation: Petiole of cell M_1 very variable in length, from subequal in length to m to quite lacking, in the latter case (including holotype) cell M_1 very deep and entirely sessile.

Basal abdominal tergite gray; tergites two to six orange, broadly blackish gray on sides, the midline very narrowly darkened; sternites and outer tergites, including hypopygium, darkened. Male hypopygium (Plate 3, fig. 31) with the tergite, 9*t*, distinct from the sternite, 9*s*. Basistyle, *b*, very large, entire, not produced into lobes or spines. Ninth tergite, 9*t*, with the caudal margin deeply notched, the median region produced

caudad into a narrow, slightly decurved point; lateral lobes of tergite with mesal flanges to appear as flattened, paddlelike blades; anterolateral portions of tergal plate produced into long arms beneath the eighth tergite. Outer dististyle, *od*, unsually small, only about one-third the length of the inner dististyle, the latter flattened, approximately parallel-sided, the outer margin at base with a small tooth. Eighth sternite, 8*s*, deeply notched medially, the sides of notch with several powerful decussate setæ, the outermost a large fasciculate bristle.

Habitat.—China-Tibet border.

Holotype, male, Yin-Kuan-Tsai, altitude 13,000 to 15,000 feet, July 25, 1930 (*Graham*). Paratypes, 2 males, Yu-Long-Gong, altitude 14,000 feet, August 14, 1930; 1 male, Yu-Long-Si, altitude 15,600 feet, July 28, 1930 (*Graham*).

Tipula variipetiolaris has the wings striped longitudinally with whitish, somewhat as in certain members of *Acutipula* or the *tricolor* group, but is very distinct in its structural details, notably of the male hypopygium. No nearly allied species is known to me.

TIPULA SUBMUTILA sp. nov. Plate 1, fig. 8.

Allied to *mutiloides;* general coloration brownish gray, the præscutum with four narrow brown stripes; frontal prolongation of head abruptly light yellow on dorsal surface; nasus distinct; antennæ with basal four segments yellow, the outer segments dark brown; femora yellowish brown, the tips dark brown; ground color of wings brown, with an incomplete pale crossband beyond cord and three more or less confluent pale areas in cells R and M surrounding the origin of Rs; R_{1+2} atrophied.

Female.—Length, about 12 millimeters; wing, 11.

Frontal prolongation of head conspicuously and abruptly light yellow on dorsal portion, the sides dark brown; nasus distinct; palpi dark brown. Antennæ with the basal four segments yellow, the succeeding segments dark brown; verticils about as long as the segment; terminal segment only a little shorter than the twelfth. Head gray laterally, broadly more infuscated on central portion.

Mesonotum obscure brownish gray, the præscutum with four narrow brown stripes, the median interspace more or less suffused; median region of scutum gray, the lobes marked with dark brown; posterior sclerites grayish brown. Pleura gray. Halteres pale, the stem yellow, the base of knob light brown, the apex more whitish. Legs with the fore coxæ gray, the

other coxæ more yellowish; trochanters light yellow; femora yellowish brown, brighter yellow basally, the tips passing to dark brown; tibiæ and basitarsi brown, the outer tarsal segments dark brown; tibial spur formula 1–2–2; claws (female) small, simple. Wings (Plate 1, fig. 8) almost as in *mutiloides;* ground color brown; three nearly equal major whitish areas in cells R and M, in the former cell lying before and beyond the origin of Rs, in the latter cell immediately beneath this origin, these areas more or less confluent and very evident; white crossband beyond cord incomplete, scarcely attaining costa, entering the base of cell M_3; a distinct pale area in outer end of cell 1st A adjoining vein 2d A. Venation: R_{1+2} atrophied; cell 1st M_2 a little longer than in *mutiloides,* cell M_1 shorter and wider.

Abdomen with ground color yellow, the tergites with a median brown stripe; sides of both tergites and sternites weakly pruinose. Ovipositor with the cerci slender, very gently upcurved, the tips subacute; hypovalvæ compressed, narrowed to the pale acute tips.

Habitat.—China-Tibet border.

Holotype, female, Yu-Long-Si, altitude 15,600 feet, August 7, 1930 (*Graham*).

Tipula submutila is most generally similar and allied to *T. mutiloides* Alexander (western China), differing in the diagnostic features above listed, especially the pattern of the præscutum, the pale dorsum of the frontal prolongation of head, yellowish brown femora, and other characters.

TIPULA JEDOENSIS sp. nov. Plate 1, fig. 9.

General coloration gray, the præscutum with four narrow brown stripes, the interspaces with conspicuous black setigerous punctures; antennæ black, the basal two segments yellow; femora with tips blackened, very broadly so on forelegs; wings whitish and brown, with a broad complete white fascia beyond cord; R_{1+2} chiefly atrophied; abdomen yellow, the tergites trivittate with dark brown, the dark areas more extensive on outer segments; ovipositor with cerci long and straight.

Female.—Length, about 14 to 15 millimeters; wing, 13 to 15.

Frontal prolongation of head black, light gray pruinose dorsally; nasus long; palpi black. Antennæ with the scape and pedicel yellow; basal portion of first flagellar segment yellow, the remainder brown; succeeding flagellar segments black; ver-

ticils not exceeding segments; terminal segment about one-third the length of the penultimate. Head brownish gray, the front and anterior orbits clearer gray; a capillary dusky line on vertex.

Mesonotum light gray, the præscutum with four narrow brown stripes, the interspaces, including the midline, with conspicuous brown setigerous punctures; median region of scutum broadly gray, the lobes marked with brown. Pleura light gray, the dorsopleural region more buffy gray. Halteres pale, the knobs dark brown. Legs with the coxæ light gray; trochanters obscure yellow; femora obscure yellow, the tips blackened, more broadly so on the forelegs where about the distal two-thirds are darkened, narrower on the other legs where only the narrow tips are blackened; tibiæ and tarsi black. Wings (Plate 1, fig. 9) with the ground color extensively whitish, variegated by light and dark brown; prearcular and costal regions yellow; a broad complete white crossband beyond cord; similar white bands before cord in outer ends of cells R and M and near wing base in cells R to 2d A, inclusive; stigma brown, confluent with a major dark area on anterior cord; other paler brown areas include the wing tip, a broad band across wing from R to margin in cell 1st A, in cases interrupted in cell Cu; veins brown, yellowish in the pale areas. Squama naked. Venation: R_{1+2} chiefly atrophied, the base more or less persistent as a weak spur without trichia; cell 1st M_2 pentagonal.

Abdomen with basal tergite gray; succeeding segments yellow with a broad, median, brown stripe and somewhat narrower lateral stripes, the dark areas more extensive and more pruinose on outer segments, restricting the ground color to small, obscure, sublateral, yellow portions. Ovipositor with cerci long, slender, and nearly straight, the margins smooth; hypovalvæ shorter and more compressed.

Habitat.—China-Tibet border.

Holotype, female, Jedo Pass, altitude 12,000 to 15,000 feet, July 17 and 18, 1930 (*Graham*). Paratopotype, 1 female; paratype, 1 female, Tatsienlu, altitude 8,000 to 9,000 feet, August 16, 1930.

The only other, approximately similar, regional species is *Tipula submutila* sp. nov., which differs in the pattern of the wings, body, and appendages. The dark setigerous punctures on the præscutal interspaces are very conspicuous and characteristic.

TIPULA MULTISTRIGATA sp. nov. Plate 1, fig. 10.

Mesonotum clear gray, the præscutum with three dark brown stripes, the median one split by a capillary pale line; median area of scutum broadly gray, the dark areas on lobes lying far laterad; wings whitish, variegated with brown, including a complete, narrow, white crossband beyond cord; R_{1+2} atrophied; R_2 very short or nearly lost by approximation of adjoining veins; M_{3+4} about one-third m; intermediate abdominal segments with the lateral margins conspicuously silvery-white.

Female.—Length, about 16 millimeters; wing, 14.

Frontal prolongation of head narrowly brownish yellow above, darker brown on sides; nasus distinct; palpi black. Antennæ with the scape obscure yellow, the basal half darkened; pedicel yellow; flagellum black; verticils exceeding segments. Head gray.

Mesonotal præscutum clear gray, with three, clearly defined, dark brown stripes, the median stripe split by a capillary pale vitta; scutum medially very broadly gray, the usual two dark areas of each scutal lobe lying far laterad; scutellum blackened, damaged in type; mediotergite gray, with a narrow dark brown median line, the posterior portion of sclerite more darkened. Pleura gray, the dorsopleural region buffy. Halteres yellow, the knobs dark brown. Legs with the coxæ gray pruinose; trochanters yellow; femora yellowish brown, the tips dark brown, relatively narrow and subequal in amount of both fore and hind legs (middle legs broken); hind femora with the central portion weakly darkened, leaving the narrow base yellow, with indications of an obscure yellow subterminal ring; tibiæ and basitarsi brownish yellow, the tips narrowly darkened; outer tarsal segments more uniformly blackened, tibial spur formula 1–?–2. Wings (Plate 1, fig. 10) whitish, with a strigate brown pattern that is arranged much as in *tetragramma* Edwards; cells C and Sc clear light yellow; a complete but narrow white crossband beyond cord; outer end of cell R_5 conspicuously white but outer end of cell R_3 uniformly darkened; cells R and M basad of origin of Rs with the dark markings much restricted in area; cell 2d A clear; veins chiefly dark brown. Squama naked. Venation: Tip of R_{1+2} atrophied; R_2 punctiform, so R_1 touches R_{2+3+4} or nearly so; Rs long, slightly exceeding twice m-cu; petiole of cell M_1 exceeding m.

Abdominal segments obscure yellow, trivittate with dark brown, on the fifth and succeeding segments the yellow coloration passing into gray; both tergites and sternites on segments

two to seven, inclusive, with the lateral margins narrowly silvery. Ovipositor with the basal shields shiny black; cerci blackish, reddish basally, relatively straight and slender, the margins smooth.

Habitat.—China-Tibet border.

Holotype, female, Yu-Long-Si, altitude 15,600 feet, August 7, 1930 (*Graham*).

Tipula multistrigata is most nearly allied to the larger and broader-winged *T. latistriga* Edwards (northeast Burma), differing in the pattern of the mesonotum and wings, in the latter with the white crossband beyond cord narrower, the white areas in cells R and M much wider. The venational details of the present form, as the loss of R_2, with cell 1st M_2 narrow and M_{3+4} unusually short, provide additional features for the separation of the two flies.

TIPULA PEDICELLARIS sp. nov. Plate 1, fig. 11; Plate 3, fig. 32.

General coloration gray, the præscutum with a median brown stripe that is trilineate with darker brown; antennæ black, the pedicel abruptly orange; tips of femora broadly blackened, most extensive on forelegs; wings whitish, sparsely patterned with pale brown, the ground color including an incomplete fascia beyond cord; R_{1+2} entire; male hypopygium with the caudal margin of ninth tergite broadly emarginate; eighth sternite with a brush of long yellow setæ.

Male.—Length, about 13 millimeters; wing, 13.2; antenna, 4.

Female.—Length, about 14 millimeters; wing, 12.2; antenna, 2.2.

Frontal prolongation of head yellowish gray above, duller laterally; nasus distinct; palpi black, the terminal segment subequal to or a little shorter than the two preceding segments combined. Antennæ black, the scape pruinose, the pedicel abruptly orange; flagellar segments (male) with weak basal enlargements; verticils shorter than the segments; terminal segment about one-third the length of the penultimate. Head brownish gray, the orbits and vertical tubercle (male) more yellowish gray, the latter weakly notched; in the female the vertical tubercle is not or scarcely developed.

Mesonotal præscutum brownish gray, the lateral stripes narrow to almost obsolete; median stripe conspicuous, the broad margins and narrower median vitta dark brown, the intermediate portions paler brown; stripe narrowed to the suture;

posterior sclerites of mesonotum clearer gray. Pleura gray, the dorsopleural region dusky. Halteres obscure yellow, more brightened at bases, the knobs dark brown. Legs with the coxæ gray pruinose; trochanters reddish yellow; femora reddish yellow basally, the tips broadly blackened, broadest on forelegs where about the basal third is pale, narrowest on posterior femora where about the distal fourth is blackened; tibiæ and basitarsi black, the proximal ends a little paler; remainder of tarsi black; tibial spur formula 1–?–2, the middle legs broken. Wings (Plate 1, fig. 11) with the ground color whitish, with a pale brown clouding over most of disk; cell Sc and prearcular region yellowish, cell C more brownish yellow; stigma darker brown; the ground areas include a narrow incomplete fascia beyond cord, extending from costa to midlength of cell M_3; a broader white fascia, more or less parallel to last-described, lies before stigma, extending across outer ends of cells R and M; cells M, Cu, and basal half of 1st A extensively whitish. Squama naked. Venation: Rs more than one-half longer than m-cu; R_{1+2} long and entire, but pale except for the extreme base, which has two or three trichia; petiole of cell M_1 nearly three times m; m-cu on M_4 just beyond origin; cell 2d A narrow.

Abdominal segments chiefly orange, trivittate with brown, the lateral margins more pruinose; outer segments and hypopygium dark brown. Male hypopygium (Plate 3, fig. 32) relatively large, with the tergite, 9*t*, sternite, 9*s*, and basistyle, *b*, entirely distinct. Ninth tergite, 9*t*, a large thin plate, pale except for the narrowly blackened margin; sclerite broad-based and parallel-sided for more than one-half the length, thence suddenly narrowed; caudal margin very broadly and gently emarginate, with indications of a low and obtuse black median tooth. Outer dististyle, *od*, flattened, pale, expanded outwardly into a spatula. Inner dististyle, *id*, with the heel portion produced into a slender rod that terminates in an acute spine. Ninth sternite, 9*s*, with a small fleshy lobule on either side of median line. Eighth sternite, 8*s*, sheathing, narrowed outwardly, the median caudal portion further produced into a broad liguliform lobe of thinner texture, this fringed with long yellow setæ.

Habitat.—China-Tibet border.

Holotype, male, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930 (*Graham*). Allotype, female, Yin-Kuan-Tsai, altitude 13,000 to 15,000 feet, July 25, 1930 (*Graham*).

Tipula pedicellaris is readily told from the other small regional species by the black antennæ, with only the pedicel

bright orange, the entire vein R_{1+2} and the structure of the male hypopygium.

LIMONIINÆ

LIMONIINI

LIMONIA (DICRANOMYIA) GRAHAMIANA sp. nov. Plate 1, fig. 12; Plate 3, fig. 33.

General coloration of thorax orange, the præscutum with a median brown line; femora yellow, the tips conspicuous dark brown; wings whitish subhyaline, the oval stigma dark brown; male hypopygium with the basistyle and ventral dististyle complicated by outgrowths, the latter with three such prolongations in the rostral region, all but the central one tufted with yellow setæ; ninth tergite with the caudal margin produced medially into a small tridentate plate.

Male.—Length, about 7.5 millimeters; wing, 9.

Rostrum brown; palpi dark brown. Antennæ black throughout; flagellar segments oval, without pedicels; terminal segment one-third longer than penultimate; verticils a little longer than the segments. Head yellowish gray; anterior vertex wider than diameter of scape.

Pronotum dark brown medially, orange-yellow on sides. Mesonotum orange-yellow, the præscutum with a conspicuous brown median line that becomes obsolete far before suture. Pleura orange-yellow. Halteres elongate, pale, the knobs weakly infuscated. Legs with the coxæ orange-yellow; trochanters and femora yellow, the tips of the latter broadly and conspicuously dark brown, the amount subequal on all legs; tibiæ yellow, the extreme bases and tips dark brown, these two areas about equal in degree; basitarsi obscure yellow, the distal third and remainder of tarsi brownish black. Wings (Plate 1, fig. 12) whitish subhyaline; stigma oval, dark brown, conspicuous; veins pale brown. Macrotrichia on veins beyond level of origin of Rs. Venation: Sc_1 ending opposite origin of Rs, Sc_2 some distance from its tip, Sc_1 being about two-thirds the length of Rs; in one wing of type, a weak adventitious crossvein in cell Sc basad of Sc_2; free tip of Sc_2 lying a short distance basad of R_2, the element subequal to R_1 alone; m-cu close to fork of M.

Abdominal tergites dark brown, the caudal margins of the segments very narrowly pale; basal sternites pale yellow, restrictedly darkened at the incisures; outer sternites infuscated; male hypopygium chiefly dark brown, the large outer lobes of the ventral dististyle abruptly white. Male hypopygium (Plate 3, fig. 33) with the median region of tergite, 9*t*, produced caudad into a small plate, the apex of which is tridentate. Basistyle,

b, with the ventromesal lobe large and complex, with a small basal and a larger apical brush of setæ. Dorsal dististyle a slender, nearly straight rod. Ventral dististyle, *vd*, large and conspicuous, the sclerotized beak portion deeply trifid, the usual two spines lying in the notch of the outer pair of arms; outer arm long and curved, gradually narrowed outwardly, with a small tuft of yellow setæ; middle arm a smooth adzlike blade; basal arm short and stout, the entire apex with a dense brush of long yellow setæ. Mesal-apical lobe of gonapophysis slender. Ædeagus long and slender, weakly setiferous on basal portion.

Habitat.—China-Tibet border.

Holotype, male, near Tang-Gu, altitute 14,000 feet, August 3 to 6, 1930 (*Graham*).

Limonia (*Dicranomyia*) *grahamiana* is named in honor of the collector of this rich series of Tipulidæ, the Reverend David C. Graham. This fly is very distinct from the now rather numerous species of the subgenus that have the male hypopygium greatly complicated by accessory outgrowths of the basistyle and ventral dististyle (as *stigmatica* Meigen, *magnicauda* Lundström, *complicata* de Meijere of the western Palæarctic region; *bifusifera* Edwards and *megacauda* of the eastern Palæarctic region; *cramptoniana* Alexander, *intricata* Alexander and *platyrostra* Alexander, of the Nearctic region). The nearest regional ally is *bifusifera* from Kashmir, which is very different in the black coloration of the body and legs, and in the details of the male hypopygium.

LIMONIA (DICRANOMYIA) PARAMORIO PLATYSOMA subsp. nov.

Male.—Length, about 5.5 millimeters; wing, 6.5.

Characters as in typical *paramorio* Alexander (eastern China, Yakushima), differing especially in an important feature of the male hypopygium. Each gonapophysis has the mesal-apical lobe very broad, the apex obtuse. In the typical form, the lobe is heavily blackened, slender, curved to the narrow acute point.

Habitat.—Japan (Kyushu).

Holotype, male, Mount Wakasugi, Chikuzen, May 3, 1931 (*Esaki et al.*).

LIMONIA (LIMONIA) EUPHILETA Alexander.

Limonia euphileta ALEXANDER, Insec. Inscit. Menst. 12 (1924) 154.
Limonia biceps ALEXANDER, Ann. & Mag. Nat. Hist. IX 15 (1925) 386–388, figs. 2–3.

The types of *euphileta* were two females from the mountains of Honshiu; the type of *biceps* was a single male from Kyushu.

It now appears from more abundant material taken in Honshiu that the type male was based on a specimen with abnormal venation. Normally, cell 1st M_2 is closed, only in abnormal specimens (but including both wings of the type of *biceps*) being open by the atrophy of m.

LIMONIA (LIMONIA) FUSCICEPS Alexander. Plate 3, fig. 34.

Limonia fusciceps ALEXANDER, Insec. Inscit. Menst. 12 (1924) 155–156.

Described from a unique female taken at Shimokebo, Hitaka, Hokkaido, Japan, August 13, 1923 (*Kuwayama*). One male and two additional females were taken on Mount Shirouma, Shinano, Honshiu, Japan, August 8, 1931 (*Machida and Nakamura*), considerably extending the range southward.

Allotype, male, Mount Shirouma, August 8, 1931. Tips of femora narrowly but conspicuously brownish black. Male hypopygium (Plate 3, fig. 34) with the caudal margin of tergite, 9*t*, very shallowly notched. Dististyle, *d*, dilated and conspicuously hairy on basal half. Gonapophyses, *g*, with the apices blackened, acute, the outer margin back from the tip with an irregularly toothed flange. Apex of ædeagus, *a*, deeply emarginate.

In the present fauna *Limonia* (*Limonia*) *tanakai* Alexander is most similar in its general appearance, having the mesonotum and legs much as in the insect discussed. The ovipositor is quite different in the two flies, being simple in *tanakai*, but with the cerci bifid at the tips in *fusciceps*.

LIMONIA (LIMONIA) PULLATA Alexander. Plate 3, fig. 35.

Limonia pullata ALEXANDER, Insec. Inscit. Menst. 12 (1924) 151–152.

The unique type, a female, was from Hinoëmata, Iwashiro, Honshiu, Japan, July 24, 1923 (*Esaki*). An additional specimen of each sex was taken at Mount Shirouma, Shinano, Honshiu, Japan, August 8, 1931 (*Machida and Nakamura*).

Allotype male, Mount Shirouma, August 8, 1931. Characters as in female, differing as follows: Pedicel of antennæ black, like the scape; basal flagellar segment almost entirely yellow. Femur with the terminal black area slightly more extensive than the yellow subterminal ring; indications of a third dark annulus on each femur just beyond midlength. Eighth and ninth tergites variegated with brown. Male hypopygium (Plate 3, fig. 35) with the caudal margin of tergite, 9*t*, weakly trilobed. Basistyle, *b*, uniformly darkened, the ventromesal lobe occupying the entire face of segment. A single dististyle, *d*, that is suddenly narrowed beyond midlength, passing into yellow and

becoming subangularly bent at this point. Gonapophyses, *g*, with the mesal-apical lobe long and slender, pale.

LIMONIA (LIMONIA) DILUTISSIMA sp. nov. Plate 1, fig. 13; Plate 3, fig. 36.

Belongs to the *flavipes* group; general coloration black, the mesonotal præscutum with the interspaces more reddish; knobs of halteres dark brown; femora obscure yellow, the tips narrowly blackened, preceded by a more or less distinct, more yellowish ring; wings pale yellow, with a diffuse, pale brown, clouded pattern; male hypopygium with the mesal-apical lobe of gonapophysis very short and obtuse at apex.

Male.—Length, 8.5 to 9.5 millimeters; wing, 10 to 11.5.

Female.—Length, 11 to 12 millimeters; wing, 11 to 12.5.

Rostrum and palpi black. Antennæ black throughout; flagellar segments subcylindrical, with the longest verticils more than twice the length of the segments and unilaterally arranged; terminal segment about one-third longer than penultimate. Head black, sparsely pruinose.

Pronotum black. Mesonotal præscutum with the ground color reddish, with heavy black median and sublateral stripes that vary in amount, the sublaterals sometimes much reduced; posterior sclerites of mesonotum chiefly brownish black or black; scutellum usually pale on caudal portion; lateral margins of mediotergite and most of pleurotergite pale. Pleura black, pruinose. Halteres obscure yellow, the knobs dark brown. Legs with the fore coxæ blackened, remaining coxæ obscure yellow, more or less darkened basally; trochanters yellow; femora obscure yellow, the tips narrowly blackened, the amount subequal on all legs, preceded by a more or less distinct clearer yellow ring; tibiæ obscure yellow, the tips narrowly darkened; tarsi chiefly dark brown. Wings (Plate 1, fig. 13) pale yellow, variegated by extensive very pale brown clouds, these including most of cells beyond cord, with other areas in basal portion of wing, as a large cloud in cell R before origin of Rs and others in the outer ends of cells Cu to 2d A, inclusive; vein Cu in cell M seamed with darker brown, especially on basal half, interrupted near outer end by a large cream-colored area; a broad band of the latter color along the cord; veins pale brown, Sc_1 and R_2 so pale as to be nearly obsolete. Venation: Sc_2 ending at near midlength of Rs, much longer than Sc_1; R_{1+2} about three times R_2 alone; m-cu close to fork of M; cell 2d A relatively narrow.

Abdomen, including hypopygium, black, the basal two or three sternites obscure yellow. Male hypopygium (Plate 3, fig. 36)

with the caudal margin of tergite, 9*t*, rounded. Basistyle, *b*, with the mesal lobe occupying the entire face and thus not appearing as a lobe. Dististyle, *d*, single, dark-colored except at the narrow base and tip. Gonapophyses, *g*, with the mesal-apical lobe unusually short and obtuse.

Habitat.—China-Tibet border.

Holotype, male, Yin-Kuan-Tsai, altitude 13,000 to 15,000 feet, July 25, 1930 (*Graham*). Allotopotype, female. Paratopotypes, 8 males and females; paratypes, 22 males and females, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930; 6 males and females, Yu-Long-Si, altitude 15,600 feet, July 28 and August 7, 1930.

Among the regional species, this fly seems to be most nearly related to *Limonia* (*Limonia*) *dilutior* Edwards, of the western Palæarctic region. It is well distinguished by the coloration of the body and legs, and by the very obtuse apices of the gonapophyses.

LIMONIA (LIMONIA) HOSTILIS sp. nov. Plate 1, fig. 14.

General coloration dark brown; tips of femora abruptly white; wings with a strong blackish tinge, without whitish areas; Sc_2 elongate, about four times Sc_1.

Sex?—Wing, 11.5 millimeters.

Rostrum and palpi brownish black. Antennæ brownish black throughout; flagellar segments oval, the longest verticils exceeding the segments and unilaterally arranged. Head dark brown.

Pronotum, mesonotum, and pleura almost uniformly dark brown. Halteres dark brown. Legs with the coxæ brown, the fore coxæ more yellowish brown; trochanters obscure yellow; femora dark brown, more brightened basally, the tips narrowly and abruptly whitened; tibiæ and tarsi dark brown; claws with a powerful acute spine on basal half, with a series of smaller serrations lying more basad. Wings (Plate 1, fig. 14) with a strong blackish tinge, the stigma only vaguely indicated and not ringed with a faint line resembling a water-mark; scarcely evident darkened clouds on Sc_2, origin of Rs, and along cord; no pale areas on wing disk; veins brown. Venation: Sc_2 elongate, approximately four times the length of Sc_1; R_2 about one-third R_{1+2}; m-cu at fork of M.

Abdomen broken.

Habitat.—China-Tibet border.

Holotype, 1 specimen, sex unknown, near Yien-Long-Shien, altitude 13,000 to 15,000 feet, August 3 to 6, 1930 (*Graham*).

The only near ally of the present fly would appear to be the larger *Limonia* (*Limonia*) *latipennis* Edwards (northeastern Burma), which is readily told by the size, very broad wings that are conspicuously variegated with whitish areas at cord and beyond stigma, and by the nearly equal veins Sc_1 and Sc_2.

LIMONIA (LIMONIA) KASHMIRICA (Edwards).

Limonia kashmirica EDWARDS, Ann. & Mag. Nat. Hist. IX 20 (1927) 226–227.

Limonia kashmirica EDWARDS, Ann. & Mag. Nat. Hist. X 1 (1928) 701–702.

Described from a female from Kashmir, altitude 11,000 to 13,000 feet. Later recorded from Tibet (Chumbitang, altitude 13,000 feet, July 25, 1924). In the Graham collection from the China-Tibet border were several additional specimens: Yin-Kuan-Tsai, altitude 13,000 to 15,000 feet, July 25, 1930; Yu-Long-Si, altitude 15,600 feet, July 28 and August 7, 1930; near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930.

The present specimens are identical with Edward's Tibetan material, but that the Kashmir type is conspecific with these Tibetan specimens is doubtful. In the present series, all but one individual are considerably larger than the type (female, length, 12 to 14 millimeters; wing, 12 to 14), and they have the anterior vertex narrow as described by Edwards for his Tibetan material, much narrower than in the Kashmir type.

In most of these specimens, there is a distinct, subterminal, black ring on the femora that is usually slightly narrower than the blackened apex but in cases is wider and very distinct, the yellow annulus inclosed being very conspicuous.

LIMONIA (LIMONIA) SYNEMPORA sp. nov. Plate 1, fig. 15.

Belongs to the *flavipes* group, allied to *kashmirica;* general coloration of mesonotum and pleura light reddish brown; wings with the ground color yellow, almost concealed by a slightly darker brown pattern, including areas at origin of Rs, fork of Sc and on R_2; Sc_1 and Sc_2 subequal; m-cu close to fork of M.

Female.—Length, about 14 millimeters; wing, 15.

Rostrum and palpi brownish black. Antennæ dark brown throughout; flagellar segments oval, with relatively short and inconspicuous verticils. Head dark brown, sparsely pruinose; anterior vertex a little wider than diameter of scape.

Pronotum and mesonotum reddish brown, with poorly defined darker markings. Pleura brownish yellow, the pleurotergite

somewhat brighter yellow. Halteres dusky, the base of knob darker, the base of stem and apex of knob yellow. Legs with the fore coxæ brown; remaining coxæ less evidently infuscated; trochanters yellow; femora yellow, the tips narrowly and abruptly brownish black, preceded by a slightly broader, clearer yellow ring that is scarcely delimited on its proximal portion; tibiæ and tarsi light brown, the terminal tarsal segments darker. Wings (Plate 1, fig. 15) with the ground color yellow, almost concealed by a very slightly darker pattern; three small, darker brown areas near costal border, lying at origin of Rs, fork of Sc and on R_2; cord and basal section of M_3 more narrowly bordered with brown; marginal areas of the ground in outer ends of cells 2d M_2 and M_3, and at ends of veins Cu_1, 1st A and 2d A. Venation: Sc_1 and Sc_2 subequal; cell 1st M_2 of moderate size, with m-cu just beyond fork of M.

Abdominal tergites brown, the bases of the segments narrowly obscure yellow; basal sternites obscure yellow, ringed caudally with brown; outer sternites yellow at base, brown laterally and caudally; genital segment obscure yellow.

Habitat.—China-Tibet border.

Holotype, female, Yu-Long-Si, altitude 15,600 feet, July 28, 1930 (*Graham*).

The only near ally of the present fly is *Limonia* (*Limonia*) *kashmirica* (Edwards), which differs especially in the pattern and venation of the wings, notably the elongate Sc_2 and distal position of m-cu.

LIMONIA (LIMONIA) IMPROVISA sp. nov. Plate 1, fig. 16; Plate 3, fig. 37.

Belongs to the *bifasciata* group; mesonotum reddish brown, the præscutum with three more or less distinct brownish black stripes; pleura dark; knobs of halteres darkened; femora yellow with two black subterminal rings; wings yellow, variegated with dark brown; stigmal area solidly darkened; R_{1+2} and R_2 subequal; male hypopygium with the dorsal dististyle a distinct blackened rod; ventral dististyle deeply split into two divergent arms or blades; gonapophyses naked at apices.

Male.—Length, 9 to 10 millimeters; wing, 12 to 13.

Female.—Length, about 10 millimeters; wing, 13.

Rostrum and palpi black. Antennæ brownish black, the pedicel obscure yellow; flagellar segments oval; terminal segment elongate, about equal to the two preceding segments combined. Head dark brown above, obscure orange beneath; anterior vertex narrow.

Mesonotal præscutum reddish brown, brownish black medially and laterally, the ground color in certain cases more restricted than in others by extensions of the dark areas; scutellum obscure yellow behind, darker basally; mediotergite yellow, with a brownish black median line that is wider at cephalic end. Pleura chiefly brownish black. Halteres pale yellow, the knobs abruptly blackened. Legs with the fore coxæ blackened; remaining coxæ and all trochanters yellow; femora yellow, with two conspicuous black rings, one postmedial, the other subterminal, the yellow apex a trifle narrower than the subterminal yellow ring; tibiæ light brown, the tips narrowly darker; tarsi somewhat darker brown. Wings (Plate 1, fig. 16) yellow, variegated with light and dark brown, somewhat as in *quadrinotata* and allies; costal border undarkened except at arculus and fork of Sc; stigma uniformly darkened; a dark area in cell R beyond arculus, with a single additional mark at midway to origin of Rs; paler brown clouds and washes in the apical and caudal cells very conspicuous; veins dark brown. Venation: Sc_1 and Sc_2 subequal; R_{1+2} and R_2 about equal; m-cu before fork of M.

Abdomen obscure yellow, the caudal margins of the segments narrowly and vaguely ringed with brown; hypopygium yellow. Male hypopygium (Plate 3, fig. 37) with the basistyle, *b*, terminating in two fleshy lobes, as in the group. Dorsal dististyle a sinuous blackened spine; ventral dististyle, *vd*, with the two portions deeply divided and widely diverging, the larger and more-flattened rostral portion with setæ. Gonapophyses, *g*, without setæ at tips.

Habitat.—China-Tibet border.

Holotype, male, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930 (*Graham*). Allotype, female, Yu-Long-Si, altitude 15,600 feet, August 7, 1930. Paratopotypes, 2 males; paratype, 1 specimen, sex unknown, with allotype.

Limonia (*Limonia*) *improvisa* is most readily told from all described allies by the solidly darkened stigmal area of the wings and by the structure of the hypopygium. The entirely separate dorsal dististyle and the outline of the ventral dististyle remind one of the conditions found in certain *Dicranomyia* species, especially in the *morio* group. In the Nearctic fauna, the fly is closest to *hudsonica* (Osten Sacken); in the Asiatic region it is but distantly allied to species such as *mendax* Alexander, *quadrinotata* (Meigen), and *yunnanica* Edwards.

ANTOCHA (ANTOCHA) FORTIDENS sp. nov. Plate 1, fig. 17; Plate 3, fig. 38.

Male.—Length, about 7.3 millimeters; wing, 8.4.

Very closely allied to *Antocha* (*Antocha*) *nebulipennis* Alexander[3] (western China), differing especially in the structure of the male hypopygium, notably the strong tooth on outer margin of outer dististyle before apex.

General coloration of thorax clear light gray, the præscutum with three conspicuous brown stripes, the median one weakly split on posterior two-thirds by a pale vitta. Knobs of halteres weakly darkened. Legs dark brown. Male hypopygium (Plate 3, fig. 38) with a strong erect spine or tooth on outer margin of outer dististyle, *od*, before the acute tip.

Habitat.—China-Tibet border.

Holotype, male, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930 (*Graham*).

PEDICIINI

DICRANOTA (RHAPHIDOLABIS) POLYMERA sp. nov. Plate 1, fig. 18; Plate 3, fig. 39.

General coloration gray, the præscutum with four narrow blackish stripes; antennæ 17-segmented, the scape and pedicel darker than the flagellum; femora yellow, with a conspicuous, nearly terminal, brown ring; wings yellow, the stigma and seams along cord and vein Cu brown; cell R_3 petiolate by presence of vein R_{2+3+4}; male hypopygium with the basistyle divided at apex into two lobes, the outer one slenderer.

Male.—Length, about 6 millimeters; wing, 7.

Rostrum gray; palpi black. Antennæ 17-segmented, relatively short; scape and pedicel black, flagellum pale brown; flagellar segments beyond basal two short-oval. Head gray.

Mesonotum dark gray, the præscutum with four narrow, more blackish stripes, the intermediate pair separated by a line that is more brownish gray than the ground. Pleura dark gray. Halteres pale, the knobs weakly darkened. Legs with the coxæ obscure brownish yellow; trochanters pale yellow; femora yellow, with a broad, dark brown, nearly terminal ring, the extreme apex a trifle paler than the ring itself; tibiæ obscure yellow, the tips narrowly infuscated; tarsi black, the proximal end of basitarsus slightly paler. Wings (Plate 1, fig. 18) relatively narrow, strongly suffused with yellow, the prearcular and costal regions clearer yellow; stigma oval, dark brown; somewhat

[3] Philip. Journ. Sci. 44 (1931) 352–353.

paler brown clouds and seams along entire length of vein Cu_1, the cord, outer medial forks, and the axilla; still paler to scarcely evident clouds at origin of Rs and tip of vein 2d A; veins pale, darker in the infuscated areas. Macrotrichia of veins long and conspicuous. Venation: Sc_1 a distance before origin of Rs about equal to three-fourths the length of the latter; R_{2+3+4} present, shorter than m-cu; R_2 longer than R_{1+2}; R_3 straight; m-cu a little exceeding the second section of M_{3+4}.

Abdominal tergites brownish black; basal sternites a trifle paler, darkened at incisures; hypopygium obscure yellow. Male hypopygium (Plate 3, fig. 39) with the apex of basistyle, *b*, deeply bifid, produced into a slender outer arm that gradually narrows to the subacute tip, the surface with relatively spare setæ, and into an inner arm that is more spatulate, provided with long conspicuous setæ, the outermost longest. A single dististyle, *d*, that is smaller than the inner lobe of basistyle but of about the same shape, densely set with blackened setæ. Interbase, *i*, a long slender blade from a slightly enlarged base. Lateral arm of tergite, 9*t*, only about one-third the size of the interbase but of nearly the same outline. Ædeagus very small.

Habitat.—Japan (Kiushiu).

Holotype, male, Wakasugiyama, Chizuzen, November 15 to 16, 1930 (*Esaki et al.*).

The present fly needs no comparison with any described *Rhaphidolabis*. The presence of 17 antennal segments marks the species as being very distinct, the range in number of antennal segments hitherto known being from 12 to 15, inclusive. The plasticity in the number of antennal segments in several genera, notably in the tribes Hexatomini and Pediciini, is becoming increasingly evident as new material becomes available from all regions of the globe.

DICRANOTA (DICRANOTA) CÆSIA sp. nov. Plate 1, fig. 19.

General coloration light gray, the præscutum with three dark brown stripes; halteres pale, the knobs dusky; legs black; wings whitish, the prearcular region light yellow; membrane with clearly defined brown spots and seams, the latter including a broad seam along vein Cu.

Female.—Length, 8.5 to 9 millimeters; wing, 9.5 to 10.

Rostrum short, gray; palpi black. Antennæ 15-segmented, black; flagellar segments short-oval, with inconspicuous verticils. Head light gray.

Mesonotal præscutum light gray, with three dark brown stripes, the median one broader, entire; lateral stripes less distinct; posterior sclerites of mesonotum dark gray. Pleura light blue-gray. Halteres pale, the knobs dusky. Legs with the coxæ pruinose; trochanters brownish yellow; remainder of legs black. Wings (Plate 1, fig. 19) whitish, the prearcular region light yellow; a conspicuous brown pattern, including spots at Sc_2 and origin of Rs, and seams along cord and vein Cu_1; vein 2d A more narrowly seamed; stigmal area paler brown than the other areas. Venation: Rs arcuated at origin; r-m variable in position, from before the fork of Rs to connecting with R_5 some distance beyond base.

Abdomen gray, the extreme caudal margin of the seventh tergite and intermediate sternites pale. Ovipositor with the cerci rather strongly upcurved, horn-colored.

Habitat.—China-Tibet border.

Holotype, female, near Tang-Gu, altitude 14,000 feet, August 3 to 6, 1930 (*Graham*). Paratopotype, a broken female.

The only allied described species is *Dicranota* (*Dicranota*) *nubecula* Edwards (northeastern Burma); this differs conspicuously in the blackish coloration of the body, which is but slightly if at all pruinose; the uniformly pale halteres; and the diffuse brown areas of the more obscure wings. The broad dark seam along vein Cu_1 is lacking in *nubecula,* while the outer medial forks have large circular brown clouds.

DICRANOTA (DICRANOTA) PALLIDIPES sp. nov. Plate 1, fig. 20.

General coloration gray, the mesonotal præscutum with three brown stripes; knobs of halteres weakly darkened; legs pale, the tips of femora, tibiæ, and basitarsi narrowly and weakly infuscated; wings whitish, with a conspicuous brown pattern that includes a broad seam on vein Cu, this becoming narrower to subobsolete on Cu_1; Rs angulated and spurred at origin; r-m subequal to basal section of R_5; cell 1st M_2 closed.

Sex?—Wing, 10.5 millimeters.

Rostrum gray; palpi black. Antennæ black, broken beyond midlength. Head gray.

Pronotum gray, dark brown medially. Mesonotal præscutum light gray, with three conspicuous dark brown stripes, the broad median vitta weakly notched at extreme cephalic end; posterior sclerites of mesonotum gray. Pleura gray. Halteres pale, the knobs weakly darkened. Legs with the coxæ pruinose; tro-

chanters obscure yellow; femora, tibiæ, and basitarsi obscure yellow, the tips narrowly pale brown; remainder of tarsi passing to darker brown. Wings (Plate 1, fig. 20) with the ground color whitish, conspicuously patterned with dark brown, the arrangement of areas almost exactly as in *cæsia*, the center of the stigmal area paler, more yellowish; a cloud at fork of M_{1+2}. Venation: Rs angulated and short-spurred at origin; R_3 gently arcuated; r-m subequal to basal section of R_5; cell 1st M_2 closed.

Habitat.—China-Tibet border.

Holotype, sex?, Yu-Long-Si, altitude 15,600 feet, August 7, 1930 (*Graham*).

Dicranota (*Dicranota*) *pallidipes* is most nearly related to *D.* (*D.*) *cæsia* sp. nov., differing especially in the coloration of the legs, and in the venation and details of pattern of the wings. These two species, with *D.* (*D.*) *nubecula* Edwards, are the largest and most conspicuous members of the subgenus in eastern Asia.

HETERANGÆUS PALLIDELLUS sp. nov. Plate 1, fig. 21.

General coloration of mesothorax pale yellow; antennæ 13-segmented, flagellum pale yellow; legs yellow, the tips of femora and tibiæ narrowly and abruptly blackened; wings whitish, with a very pale brown pattern, arranged chiefly as broken crossbands; cell C chiefly pale, sparsely variegated by dark spots and dots; Rs weakly angulated, not spurred; supernumerary crossvein in cell R_4 in approximate transverse alignment with R_2.

Female.—Length, 11 to 12 millimeters; wing, 9 to 9.5

Rostrum and palpi brownish black. Antennæ 13-segmented; scape and pedicel dark brown, flagellum light yellow; flagellar segments short, each with one appressed seta that is more than twice the length of the segment alone. Head brownish gray, clearer on the orbits.

Mesonotum and pleura almost uniformly pale yellow, the præscutum with an ill-defined median dark stripe. Halteres pale. Legs yellow, the tips of femora and tibiæ conspicuously blackened; outer tarsal segments blackened. Wings (Plate 1, fig. 21) whitish, with a very pale brown pattern, arranged chiefly as broken crossbands at level of origin of Rs, along cord and across the outer series of supernumerary crossveins; cell C pale, variegated by small brown spots; dark area at origin of Rs only slightly involving cell C; transverse dark areas in cell 1st A lacking or few in number. Venation: Rs weakly

angulated but not spurred at origin; Sc_2 some distance before origin of Rs; supernumerary crossvein in cell R_4 in approximate transverse alignment with R_2; venation of radial field more or less variable, r-m either connecting directly with fork of Rs or up to one-third its length beyond this fork; supernumerary crossvein in cell M_1 at or before midlength of cell.

Abdomen with basal segments yellow, beyond the third passing into dark brown. Ovipositor with the compressed cerci upcurved, blackened basally.

Habitat.—Japan (Honshiu).

Holotype, female, Mount Kurobegoro, Echiu, in subalpine meadow, altitude 7,800 feet, August 8, 1931 (*Imanishi*). Paratopotype, female.

The only other *Heterangæus* with cell C pale, variegated by darker, is *H. gloriosus* (Alexander), of Saghalien. This differs most evidently in the dark wing pattern, with cells C and 1st A more extensively involved, and in the venation, as the long-spurred Rs and the distal position of the supernumerary crossvein in cell R_4.

HETERANGÆUS JAPONICUS CRENATUS subsp. nov.

Male.—Length, about 8 millimeters; wing, 8.

Generally similar to typical *japonicus* (Alexander), differing as follows: Halteres shorter, if bent backward ending shortly beyond midlength of abdominal tergite two; in typical *japonicus* the halteres are unusually long and slender, if bent backward extending to caudal margin of tergite two or nearly so. Wings with the dark pattern broader, restricting the white ground, the dark color deep and more intense. Wings broader, the posterior border strongly crenate, cell 2d A being much wider and more deeply incised than in the typical form.

Habitat.—Japan (Honshiu).

Holotype, male, Mount Ohdai, Nara, June 5, 1930 (*Sakaguchi*).

ULA KIUSHIUENSIS sp. nov. Plate 1, fig. 22; Plate 3, fig. 40.

General coloration of mesonotal præscutum reddish brown, darker medially; postnotum and pleura darker reddish brown; knobs of halteres infuscated; wings with stigmal area almost uniformly darkened, with only a minute paler central area; cell 1st M_2 relatively large, subequal in length to vein M_4; male hypopygium brownish black, the dististyle arcuate, with seven or eight blackened spines that are scattered, some extending down mesal face of style to near midlength.

Male.—Length, about 5.5 millimeters; wing, 6.3.

Rostrum and palpi black. Antennæ black throughout, in male elongate, if bent backward extending to shortly beyond base of abdomen; flagellar segments subcylindrical, with verticils that are shorter than the segments. Head blackish, sparsely pruinose.

Mesonotum reddish brown, the præscutum darker brown medially; postnotum dark reddish brown. Pleura reddish brown. Halteres pale, the knobs dark brown. Legs with the coxæ and trochanters yellow; remainder of legs obscure yellow, the tips of femora, tibiæ, and outer segments of tarsi infuscated. Wings (Plate 1, fig. 22) with a strong brown tinge; stigma almost uniformly darker brown, only the central portion restrictedly paler; a dusky cloud on r-m; scarcely evident smaller clouds on Sc_2 and origin of Rs; veins brown. Venation: Basal section of R_5 eliminated, r-m being at the fork of Rs; cell 1st M_2 subequal in length to vein M_4.

Abdominal tergites uniformly brownish black; sternites obscure yellow, infuscated laterally; hypopygium brownish black. Male hypopygium (Plate 3, fig. 40) with the caudal margin of tergite, 9*t*, evenly and convexly rounded, with a marginal series of long setæ. Dististyle, *d*, elongate and slightly arcuate, armed with seven or eight spines that continue down the mesal face of style. What appears to be a lateral tergal arm lies at the lateral portion of the tergite, just mesad of the basistyle and appears as a slender sinuous spine from a dilated base, about the distal half of this spine paling to white.

Habitat.—Japan (Kiushiu).

Holotype, male, Sobosan, Bungo, August 8, 1931 (*K. Yasumatsu*).

Ula kiushiuensis is most generally similar to *U. succincta* Alexander (Honshiu), differing most evidently in the distinct structure of the male hypopygium.

ILLUSTRATIONS

[*a*, Ædeagus; *b*, basistyle; *d*, dististyle; *g*, gonapophysis; *i*, interbase; *id*, inner dististyle; *od*, outer dististyle; *p*, phallosome; *s*, sternite; *t*, tergite; *vd*, ventral dististyle.]

Plate 1

Fig. 1. *Tipula* (*Schummelia*) *esakiana* sp. nov., venation.
2. *Tipula* (*Schummelia*) *honorifica* sp. nov., venation.
3. *Tipula* (*Acutipula*) *biramosa* sp. nov., venation.
4. *Tipula* (*Vestiplex*) *grahami* sp. nov., venation.
5. *Tipula* (*Lunatipula*) *transfixa* sp. nov., venation.
6. *Tipula oreada* sp. nov., venation.
7. *Tipula variipetiolaris* sp. nov., venation.
8. *Tipula submutila* sp. nov., venation.
9. *Tipula jedoensis* sp. nov., venation.
10. *Tipula multistrigata* sp. nov., venation.
11. *Tipula pedicellaris* sp. nov., venation.
12. *Limonia* (*Dicranomyia*) *grahamiana* sp. nov., venation.
13. *Limonia* (*Limonia*) *dilutissima* sp. nov., venation.
14. *Limonia* (*Limonia*) *hostilis* sp. nov., venation.
15. *Limonia* (*Limonia*) *synempora* sp. nov., venation.
16. *Limonia* (*Limonia*) *improvisa* sp. nov., venation.
17. *Antocha* (*Antocha*) *fortidens* sp. nov., venation.
18. *Dicranota* (*Rhaphidolabis*) *polymera* sp. nov., venation.
19. *Dicranota* (*Dicranota*) *cæsia* sp. nov., venation.
20. *Dicranota* (*Dicranota*) *pallidipes* sp. nov., venation.
21. *Heterangæus pallidellus* sp. nov., venation.
22. *Ula kiushiuensis* sp. nov., venation.

Plate 2

Fig. 23. *Tipula* (*Schummelia*) *esakiana* sp. nov., male hypopygium, details.
24. *Tipula* (*Schummelia*) *honorifica* sp. nov., male hypopygium, details.
25. *Tipula* (*Acutipula*) *incorrupta* sp. nov., male hypopygium, details.
26. *Tipula* (*Acutipula*) *biramosa* sp. nov., male hypopygium, details.
27. *Tipula* (*Acutipula*) *biramosa* sp. nov., male hypopygium, dististyles.
28. *Tipula* (*Vestiplex*) *grahami* sp. nov., male hypopygium, details.
29. *Tipula* (*Lunatipula*) *transfixa* sp. nov., male hypopygium, details.
30. *Tipula oreada* sp. nov., male hypopygium, details.

Plate 3

Fig. 31. *Tipula variipetiolaris* sp. nov., male hypopygium, details.
32. *Tipula pedicellaris* sp. nov., male hypopygium, details.
33. *Limonia* (*Dicranomyia*) *grahamiana* sp. nov., male hypopygium.

FIG. 34. *Limonia* (*Limonia*) *fusciceps* Alexander, male hypopygium.
35. *Limonia* (*Limonia*) *pullata* Alexander, male hypopygium.
36. *Limonia* (*Limonia*) *dilutissima* sp. nov., male hypopygium.
37. *Limonia* (*Limonia*) *improvisa* sp. nov., male hypopygium.
38. *Antocha* (*Antocha*) *fortidens* sp. nov., male hypopygium.
39. *Dicranota* (*Rhaphidolabis*) *polymera* sp. nov., male hypopygium.
40. *Ula kiushiuensis* sp. nov., male hypopygium.

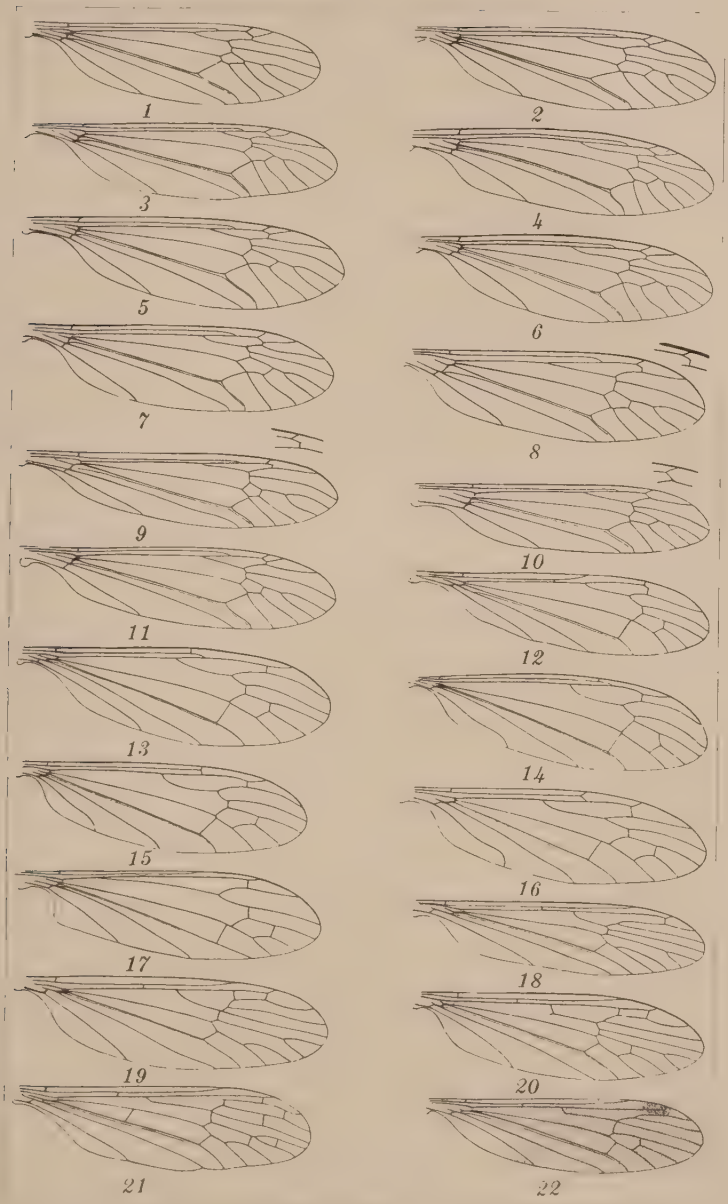

PLATE 1.

9t
9t
b
8s
9s
id
23
9t
od
id
8s
9s-b
9t
24
9t
id
9s
b
8s
8s
9t
25
9t
od
id
b
9t
8s
9s
8s
26
9t
od
id
9s
b
8s
9t
b
9s
8s
28
9t
id
9t
b
8s
id
9s
29
id
od
9t
d
b
8s
9s
27
p
30a
9t
d
8s
30b

PLATE 2.

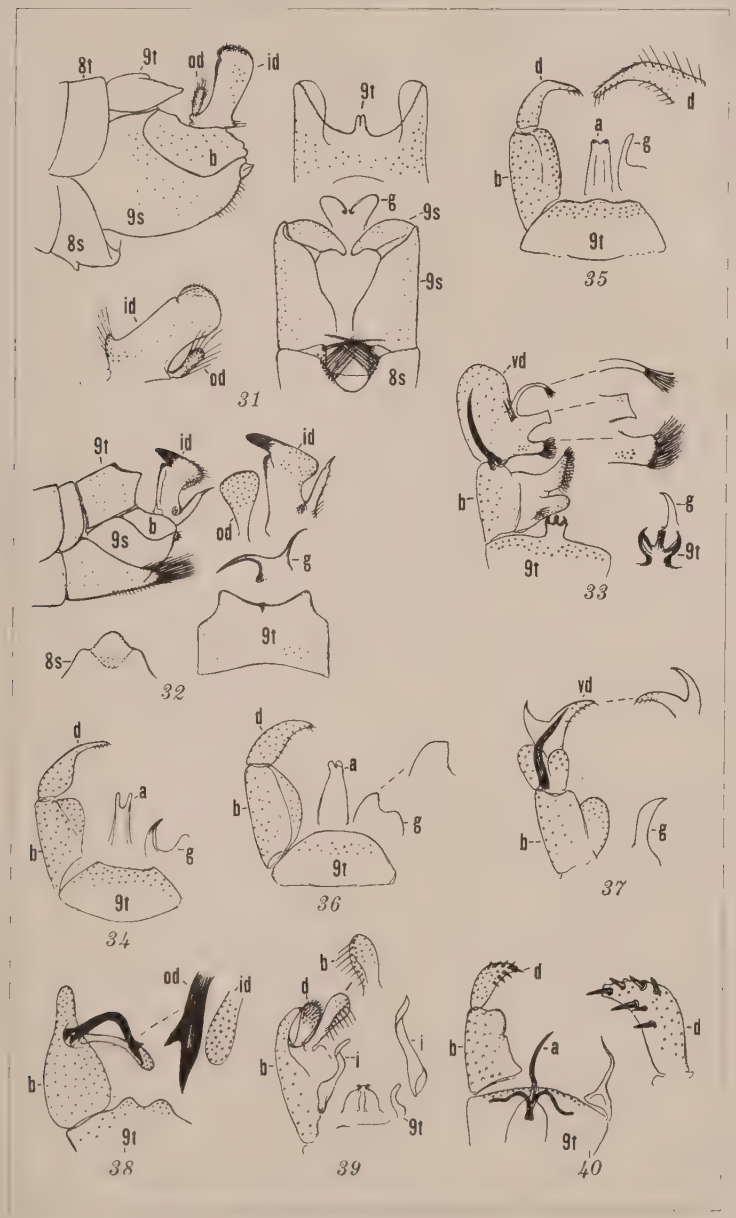
8t
9t
od
id
b
9s
8s
31
9t
g
9s
9s
8s
d
d
a
g
b
9t
35
vd
b
g
9t
9t
33
9t
id
id
9s
b
od
g
9t
8s
32
d
a
b
g
9t
34
d
a
b
g
9t
36
vd
b
g
37
od
id
b
9t
38
b
d
i
i
b
9t
39
d
d
b
a
9t
40

PLATE 3.

DESCRIPTIONS OF A NEW GENUS AND ELEVEN NEW SPECIES OF EUPTERYGINÆ (HOMOPTERA) FROM THE PHILIPPINE REGION

By W. L. McAtee

Of the United States Biological Survey, Washington

Most of the specimens upon which this paper is based were sent to me by the late Dr. C. F. Baker and the types are in my collection; the remainder are from the Baker collection now in the United States National Museum. It is suggestive of the wealth of species of the region that nearly every specimen proved distinct on the basis of genital characters. Possibly some species have been described twice on account of present inability to associate the sexes, but settlement of this point must await the accumulation of richer material.

Genus SIROSOMA novum

Venation of both tegmen and wing as in *Erythroneura* but distinct basally in the tegmen; claval vein also evident. The chief distinguishing character is the presence of coarse subcontiguous punctures (relative to the size of the insect they are huge pits) on the clavus, base of corium, pronotum, and head. Those on the body overlie pale pigment spots, and grade into similar maculations apparently not depressed that occur on nearly all parts of the body surface in one species or another.

Type of the genus *Sirosoma hiaticula* sp. nov. here described.

Two previously described groups of Eupteryginæ are said to possess conspicuous punctures. One is *Kybos* Fieber, usually considered a subgenus of *Empoasca* Walsh, but the punctures it possesses are insignificant compared to those characterizing *Sirosoma*. The genus *Aidola* Melichar is described as having the base of clavus and corium with large deep punctures. Nothing is said as to punctures on the head and thorax, and the venation also apparently differs from that of *Sirosoma*, in having only two instead of three sectors of the wing approaching the apical margin.

Key to the species of Sirosoma.

MALES.

a. Outer claspers narrowed subbasally.

b. Inner margins of claspers sinuate near base bounding a prominent elliptical hiatus; lower profile much less than a quarter circle; basal triangles of scutellum and two dots on vertex dark brown; tegmina generally fumose without vittæ..... *S. hiaticula* sp. nov.

bb. Inner margins of claspers parallel; lower profile of claspers describing nearly a quarter circle, the apices much upturned; basal triangles of scutellum black, a faint narrow dusky vitta traversing the tegmen behind each of these spots; no dots on vertex.

S. arcuata sp. nov.

aa. Outer claspers not narrowed subbasally.

c. Outer claspers conjointly forming a broad flattened sheath, through an opening near the end of which the apices of the inner claspers can be seen; a single bristle on each laterad of this opening; general color fumose, basal triangles of scutellum, a wedge and two dots at middle of vertex, the clypeus, and a vitta each side of face above it dark brown..... *S. saccula* sp. nov.

cc. Outer claspers otherwise.

d. Outer claspers large, pronotum without black dots.

e. Outer claspers inflated basally, pinched in apically, the apices acute, without median groove; basal triangles of scutellum castaneous, a broad, fumose vitta on each tegmen; no markings on head ..... *S. abrupta* sp. nov.

ee. Outer claspers not inflated basally, tapering gradually, the inner margins jointly forming a percurrent longitudinal groove; basal triangles of scutellum black, a narrow fumose vitta behind each on tegmen, two dots on vertex dusky, and median vitta on face black..... *S. canalicula* sp. nov.

dd. Outer claspers small, pronotum with black dots.

f. Claspers nearly as long as hind tarsus, dark mark at base of corium short ..... *S. maquilinga* sp. nov.

ff. Claspers distinctly shorter than hind tarsus, mark at base of corium an elongate vitta..... *S. bakeri* sp. nov.

FEMALES.

g. Genital plate with a median emargination.

h. Genital plate more than half as long as ovipositor, posterior margin rounded with a slight median emargination; basal triangles of scutellum, two dots on vertex, and two short vittæ below middle of face brown; pronotum brownish, tegmen fumose..... *S. batilla* sp. nov.

hh. Genital plate much less than half as long as ovipositor, with a profound emargination.

i. Emargination U-shaped; scutellum pale, two black dots on pronotum near hind margin, and a black dash on base of corium.

S. maquilinga sp. nov.

ii. Emargination V-shaped; basal triangles of scutellum black; disk of pronotum and of face brown, and faint broad vitta on tegmen dusky.
S. emarginata sp. nov.

gg. Genital plate with a median projection, each side of which is rounded emarginate.

j. Median part of hind margin of genital plate broadly rounded; basal triangles of scutellum and oval spot on lower part of face black; tegmina white.
S. frontalis sp. nov.

jj. Median part of hind margin of genital plate in the form of a short produced, thickened, almost knob-like, process; no spots on scutellum or head; tegmina greenish yellow on inner half.
S. clavata sp. nov.

SIROSOMA HIATICULA sp. nov.

General color above yellowish fumose; basal triangles of scutellum, and a roundish dot each side of and near middle of vertex, dark brown; face pale brownish except peripherally; pleura and dorsum of abdomen dark brown (nearly black); venter pale brownish.

Vertex rounded, rather short, but little longer at middle than along eye; coarse punctures restricted to basal half of clavus and corium, rather evenly distributed over scutellum (except basal triangles), pronotum, and pleura, except that there is a smooth area between front and mid coxæ; on the head there is an oval group on middle of face connected upwardly with a dense group covering upper part of face and the vertex, from which a tract extends also over the disk of each cheek; abdomen pale dotted both above and below. Outer claspers as described in key. Length 3.5 millimeters.

Holotype male, Sandakan, Borneo.

SIROSOMA ARCUATA sp. nov.

General color of head and thorax above pale ivory, a semicircular mark connected with the black basal triangles of scutellum showing duskily through the pronotum; tegmen whitish hyaline with a narrow dusky percurrent vitta paralleling inner margin; underparts stramineous, somewhat sordid on face.

Vertex moderately long, rounded, but little longer at middle than along eye; coarse punctures restricted to basal half of clavus and corium; generally prevalent on scutellum (except basal triangles), and on pronotum except near hind margin; a row near hind margin of vertex, large groups on the disk of face, and each cheek, the head otherwise smooth; pleura and dorsum

pale dotted, venter only obscurely so if at all. Outer claspers as described in key. Length 4 millimeters.

Holotype male, Mount Maquiling, Luzon.

SIROSOMA SACCULA sp. nov.

Vertex ivory with a median black wedge from hind margin projecting between two ovoid black spots; pronotum mostly livid, anterior margin ivory; scutellum livid, the basal triangles brown; tegmina fumose; face ivory, clypeus and a triangular marking above it each side of front; abdomen slaty both above and below; legs and genitalia stramineous.

Vertex rounded, rather short, about one-third longer at middle than next to eye; coarse punctures restricted to basal half of clavus and basal fourth of corium; scutellum coarse-punctate except for apex and basal triangles; pronotum generally coarse-punctate; punctures conspicuous on cheeks, sparse but discernible elsewhere on head; pleura, and abdomen both above and below, pale dotted. Outer claspers as described in key. Length 3.5 millimeters.

Holotype male, Sandakan, Borneo (United States National Museum).

SIROSOMA ABRUPTA sp. nov.

General color of head and thorax above yellowish fumose, basal triangles of scutellum castaneous; tegmen whitish hyaline with a broad, median percurrent, fumose, longitudinal vitta; lower parts stramineous, most of face with a pale yellowish brown wash.

Vertex rounded, moderately long, about one and one-half times as long at middle as along inner margin of eye; coarse punctures restricted to basal half of scutellum and basal fourth of corium; generally distributed on scutellum except basal triangles, and on pronotum except for a rather broad posterior edging moderately expanded anteriorly on each side of middle; those on head distributed as in the last species, *S. arcuata,* but more sparsely; punctures evident on pleura; abdomen with pale pigment dots both above and below. Outer claspers as described in key. Length 3.5 millimeters.

Holotype male, Sandakan, Borneo.

SIROSOMA CANALICULA sp. nov.

General color of head and thorax above pale yellowish, a dusky triangular marking connected with the black basal triangles of scutellum showing through pronotum; a dark brown elliptical dot on each side about midway between middle of vertex and

eye; underparts somewhat sordid stramineous, face with a broad median blackish brown vitta, narrowed above; dorsum of abdomen dark brown.

Vertex rounded, rather short, only a little longer at middle than along inner margin of eyes; coarse punctures restricted to basal fourth of corium, but occurring over nearly all of clavus; dotting scutellum except basal triangles; disk and broad hind margin of pronotum polished, the punctures on anterior portion exceptionally large; head with sparse coarse punctures on vertex, middle of face, and disk of cheeks, otherwise polished; pleura punctate, abdomen pale dotted above. Outer claspers as described in key. Length 5 millimeters.

Holotype male, Los Baños, Luzon.

SIROSOMA MAQUILINGA sp. nov.

Color above stramineous washed with greenish yellow anteriorly; pronotum with large black spot near hind margin on each side in front of anterior angle of scutellum; tegmen with a short black dash in middle of corium near base; extreme apex of scutellum and disk of tergum black in male; lower parts stramineous; breast and apices of claspers black in male; ovipositor of female brown.

Vertex rounded, short, nearly the same length at all points; coarse punctures visible on basal half of clavus and basal fifth of tegmen; conspicuous on scutellum except basal triangles and apex which are polished; punctures very coarse and generally distributed on pronotum except along hind margin; head polished so that punctures are obsolete except on cheeks; pleura punctate, and pale dots visible on dark tergum of male; not evident on pale portions of abdomen; genitalia as described in key. Length 3 to 3.5 millimeters.

Holotype male and allotype female, Mount Maquiling, Luzon (United States National Museum).

SIROSOMA BAKERI sp. nov.

Head and thorax above stramineous, tegmina whitish hyaline; pronotum with two black dots posteriorly as in *maquilinga;* a tiny dash on edge of scutellum behind each of them; dusky vitta on corium about two-thirds as long as clavus, dense basally, evanescent distally; lower parts stramineous.

Vertex short, rounded, of about equal length at all points; distribution of coarse punctures as in *maquilinga.* Length 3.25 millimeters.

Holotype male, Mount Maquiling, Luzon (United States National Museum).

SIROSOMA BATILLA sp. nov.

General color of head and thorax above fulvous, pronotum with some brown flecks anterolaterally, and with a dark horseshoe-shaped marking connecting posteriorly with the black scutellar triangles dimly showing through; vertex with a pair of large roundish polished black spots near together at middle; tegmina fumose; face varying from stramineous on cheeks to ivory at middle and fulvous near vertex, with a short dark brown vitta each side of middle above clypeus; legs and genital plate stramineous; abdomen dark brown both above and below.

Vertex rounded, moderately long, of about same length at all points; coarse punctures chiefly restricted to basal half of clavus and to basal fourth of corium; they or the pigment dots about evenly distributed over scutellum except apex and basal triangles, pronotum, vertex, and face, pleura, and abdomen both above and below; a few are present even on the ovipositor sheath; genital plate like an inverted scoop, stramineous, with a brownish lunate depression near base, otherwise as described in key. Length 3.5 millimeters.

Holotype female, Singapore.

SIROSOMA EMARGINATA sp. nov.

General color of head and thorax above stramineous, vertex with a faint dusky point each side near middle; pronotum except broad anterior margin underlaid by fuscous; scutellar triangles black, disk fumose; clavi within claval veins greenish white, tegmen fumose with broad costal margin and a longitudinal streak just exterior to apex of clavus, hyaline; face stramineous, disk brownish; legs stramineous, abdomen dark brown both above and below.

Vertex rounded, about one and one-half times longer at middle than next to eye; coarse punctures mostly restricted to basal half of clavus, and basal fourth of corium, and disk of scutellum; pronotum generally coarse punctate except along hind margin; head coarse punctate except for apex, clypeus, and lateral margins of cheeks; pleura coarse punctate and abdomen pale dotted both above and below; genital plate as described in key. Length 4 millimeters.

Holotype female, Sandakan, Borneo.

SIROSOMA FRONTALIS sp. nov.

General color of head and thorax above stramineous, a pair of faint brownish dots on vertex near middle; small basal triangles of scutellum black; tegmina whitish hyaline; face stramineous with a median oval, dark brown spot just above clypeus; legs, venter, and connexivum stramineous; disk of dorsal surface of abdomen brownish.

Vertex rounded, rather short, of about same length throughout; coarse punctures distributed over whole clavus, and basal half of corium, disk of scutellum, all of pronotum except narrow hind margin; cheeks densely coarse punctate, a few punctures along each side of middle of face, and along hind margin of vertex, head otherwise nearly smooth; abdomen without pale dots; genital plate as described in key. Length 4 millimeters.

Holotype female, Basilan Island.

SIROSOMA CLAVATA sp. nov.

Head and thorax above uniform honey-colored; tegmina whitish hyaline with a brassy wash over clavus and adjacent corium, somewhat fumose apically; underparts honey-colored, legs paler, disk of dorsum of abdomen brownish.

Vertex rounded, moderately long, about one and one-half times as long at middle as along eye; coarse punctures restricted to basal half of clavus, basal fifth of corium, and disk of scutellum; pronotum coarse punctate except for broad hind margin; coarse punctures (or their analogues) present over most of head (except rondure from vertex to face) but difficult to see; pleura punctate; pigment dots visible on dark, but not on other, portions of abdomen. Genital plate as described in key. Length 4 millimeters.

Holotype female, Sandakan, Borneo.

TERMINOLOGY USED FOR ANOPHELES OF THE FUNESTUS-MINIMUS SUBGROUP IN RECENT PAPERS BY RUSSELL AND OTHERS

By Paul F. Russell

Member of Field Staff of the International Health Division the Rockefeller Foundation

King(1) has recently published a detailed study of the *funestus-minimus* subgroup of *Anopheles* which he now considers to include in the Philippines three species; namely, *A. minimus* var. *flavirostris* Ludlow 1914, *A. mangyanus* Banks 1907, and *A. filipinae* Manalang 1930. This new arrangement of the very important *funestus-minimus* subgroup appears to meet the situation better than any previous study and is likely to become the accepted classification. Therefore, it seems advisable to write these notes based on a reconsideration of specimens reported by the author and his colleagues in some publications which were in manuscript prior to King's final conclusions.

In the first report by Russell(2) on daytime resting places of adult anophelines, "*Anopheles minimus* (Theobald 1901)" of Tables 3 and 4, page 646, includes both *Anopheles minimus* var. *flavirostris* Ludlow and *Anopheles mangyanus* Banks as described by King.(1) In the second report by Russell(3) "*A. minimus*" referred to throughout the report is *A. minimus* var. *flavirostris* of King.(1)

In a paper by Russell(4) regarding larva control by stranding and flushing, "*A. minimus*" includes the *funestus-minimus* subgroup of King.(1) In a paper by Russell and Santiago(5) the *A. minimus* larvæ referred to as having been found in wells were *A. minimus* var. *flavirostris* of King.(1)

Finally, in a paper by Holt and Russell,(6) reporting malaria and *Anopheles* reconnaissance, wherever the term "*minimus* group" has been used King's(1) term of "*funestus-minimus* subgroup" may be substituted. Wherever the name "*A. minimus*" is used, *A. minimus* var. *flavirostris* may be understood.

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THE CAST NET AS A DEEP-WATER FISHING APPLIANCE IN MANILA BAY *

By AGUSTIN F. UMALI

Of the Fish and Game Administration, Manila

THREE PLATES AND SIX TEXT FIGURES

INTRODUCTION

The cast net, locally known as *dala* in Tagalog, *laya* in Visayan and Bicol, *tabocol* in Ilocano, *sabucol* in Pangasinan, *lala* in Zambal, and *atarraya* in Tao Sug and Samal, is perhaps the oldest type of fishing net known to man. It is especially interesting in that the hand net used by the apostles during Biblical times as mentioned in the New Testament is said to be one similar to the present-day cast net. It is a net which has been universally in use since time immemorial.

In the Philippines, this net is the kind generally used by both the Christian and the Mohammedan Filipinos. Its commonest use, of which much has been written, is along estuaries, banks of rivers and lakes, and wading depths on sea beaches where an individual fisherman with a net and a basket for the catch plies his trade on a small scale. A fisherman stealthily wading in a stooping position, spying the presence of fish and ever ready to cast his net over his unsuspecting victims, is a common sight near fishing villages.

Less frequently observed is the use of the dala in deep water. Here its extensive utilization has necessitated an increase in its size and the employment of a boat in connection with its operation. The use of this net in deeper waters, especially popular among the native fishermen of Rizal and Bataan where fleets of such outfits are seasonally observed, is the subject of the present discussion.

THE FISHING GROUND

The sphere of operation of this fleet of dala fishermen from Rizal and Bataan is the open waters of Manila Bay, where there

* Contribution No. 1 from the Fish and Game Administration, Department of Agriculture and Commerce.

are vast schools of herrings and other pelagic species. As the boats used are small, they seldom venture into the open sea. Any bay or gulf, which is the natural abode of pelagic species that move in shoals, is a favorable fishing ground for the operation of this gear.

THE BOATS

The boats employed are ordinary dugouts or bancas of the outrigger type with an average capacity of about one-half ton net (Plate 1, fig. 1). They are about 30 feet in length with a width of 3 feet and a depth of 2 feet. Each boat is rowed by from three to four oarsmen, and when the wind is favorable, a sail is hoisted to the top of a detachable mast.

In the prow of the boat is a somewhat elevated, rectangular, wooden platform about 3 feet long and about 2 feet wide (Plate 1, fig. 2). This serves as a stage for the dala thrower when he casts the net. Behind the forward crosspiece of the outrigger is a bamboo pole about 5 feet high, which is provided with a curved end that forms a hook from which the net with the gilled fish is hung for the convenience of the fishermen in removing the catch from its meshes. A support is provided for the detachable wooden mast where the latter is stepped well forward at a point just aft of the platform.

The anterior third of the hold of the boat behind the platform, which is provided with some sort of flooring, serves as the working place for the mending of the nets, the picking and sorting of the catch, and the storing of the reserve nets and the catch. The central portion of the boat is for the rowers, each of them being provided with one oar and a paddle—the former is used when reconnoitering for schools of fish, and the latter is employed when approaching the school with caution and perfect quiet in order not to frighten the fish into diving.

In the stern is the place of the steersman, who is provided with a special paddle, as the boat has no rudder. Behind the place of the steersman is another bamboo pole of almost the same size and length as the one in the bow which, is conjunction with the latter, serves to make secure the awning spread as a protection against the sun.

THE FISHERMEN

A dala fishing outfit of this type is manned by from five to seven fishermen. One of them is an expert in the art of casting the net and at the same time skilled in the differentiation of schools of fish by the disturbance created on the surface of

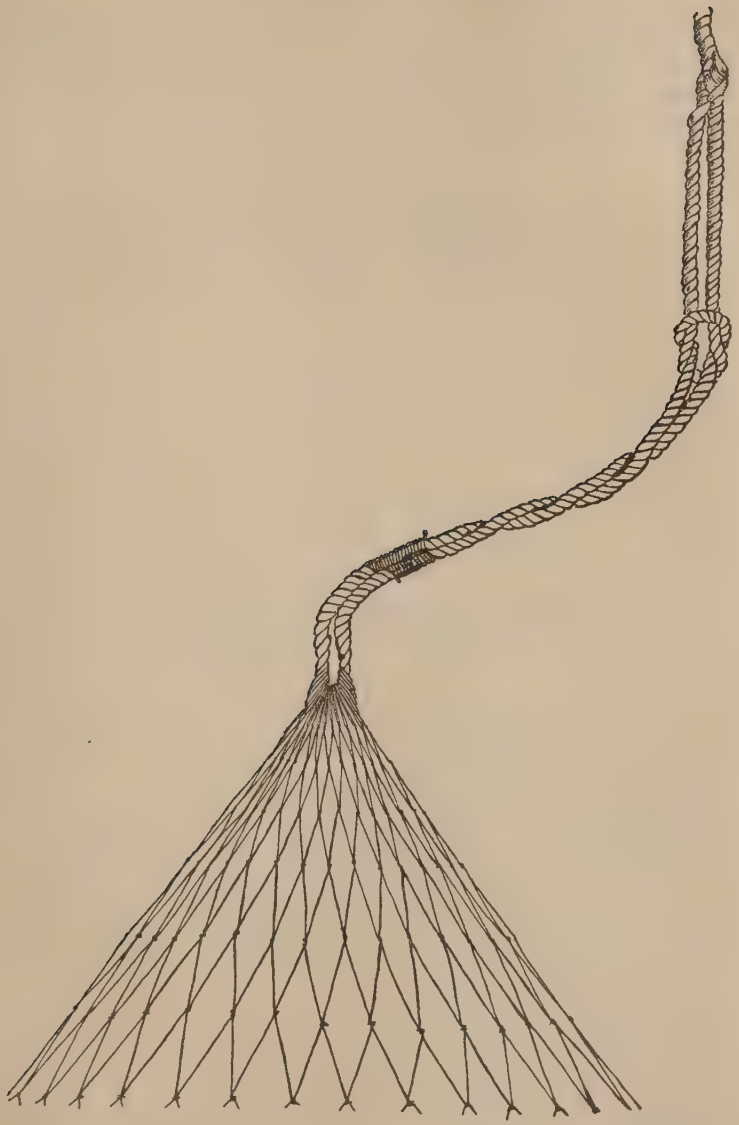

FIG. 1. The apical portion of a typical dala, showing attachment of hauling-in line.

the water as they swim about. Another member of the crew is the steersman, whose main work is to direct the course of the vessel according to instructions from the expert thrower. The rest are the oarsmen, who row the boat and also help in picking the gilled fish from the meshes of the net.

THE FISHING GEAR

The dala is a circular net, heavily weighted with lead sinkers around its lower edge and provided with a hauling-in line attached to the apical portion. When distended, it has the form of a cone; when thrown into the water, it settles like an open

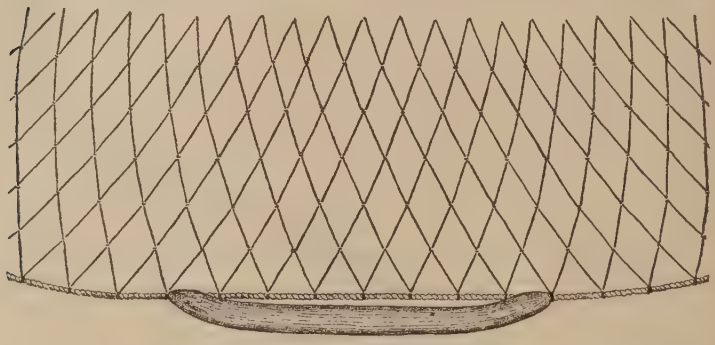

FIG. 2. Portion of weighted edge of the dala, showing the way the lead sinkers are attached.

umbrella, and when it is drawn out by pulling on the main cord, the sinkers gather together giving the net the appearance of a closed or collapsed umbrella.

The cast net has two main parts; namely, the hauling-in line and the net proper. The line, which varies in length from 40 to 60 feet, is a $\frac{1}{4}$-inch [1] cotton string. In the typical dala, (text fig. 1), it is directly attached to the apical line that supports the webbing of the apex of the supposedly conelike net. It acts as a retrieving line in the actual casting operation. The greater portion of its length is coiled on the elevated platform when the net is still uncast.

The net itself is the main portion of the gear that either impounds or more generally gills the fishes. It is about 30 feet high with a diameter of about 25 feet at the base. The meshes of the web and the size and kind of the twine used are dependent upon the size and kind of fish for which the net is espe-

[1] The measurements of the lines in this work are the diameters.

cially constructed. The netting of the upper two-thirds of the majority of the dala observed is of No. 50 linen thread and the lower one-third of No. 40. As mentioned in a foregoing paragraph, the lower edge is heavily weighted with lead sinkers strung at regular intervals (text fig. 2). These weights, which are cylindrical, are about 4¼ inches long and about ¼ inch in diameter; each sinker weighs about 1½ ounces and is provided with two perforated projections that serve to hold it to the edge of the net.

Of a rather different make is the *pangduhay*, the dala used for catching pomfrets (*duhay*) *Stromateus niger* (Bloch). In addition to the net proper, in this case of China-grass [*Boehmeria nivea* (Linn.) Gaudich.] twine, there are about twenty baited lines (text fig. 3), each of ⅜-inch cotton rope about 25 feet long, provided with a wooden float at one end while to the opposite end, which is weighted with a stone, are attached several jellyfishes that serve as the bait. These accessory parts are used to attract the pomfrets upon which the net is cast. Another accessory implement is the *pamangkao*, a long-handled scoop (text fig. 4), which is used for scooping and raising the baited lines from the water when determining whether or not the pomfrets have been attracted. The handle is a bamboo pole about 15 feet long, while the scoop proper is a netting of No. 20 cotton twine webbed with a mesh of 1 inch stretched that is

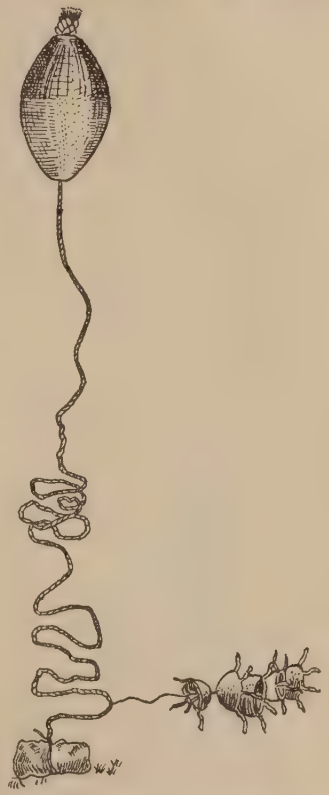

FIG. 3. A baited line, an accessory part of a panduhay.

FIG. 4. The pamangkao, a long-handled scoop.

laced to an iron ring about 9 inches in diameter. While in the other types of dala the apex is closed and the hauling-in line is

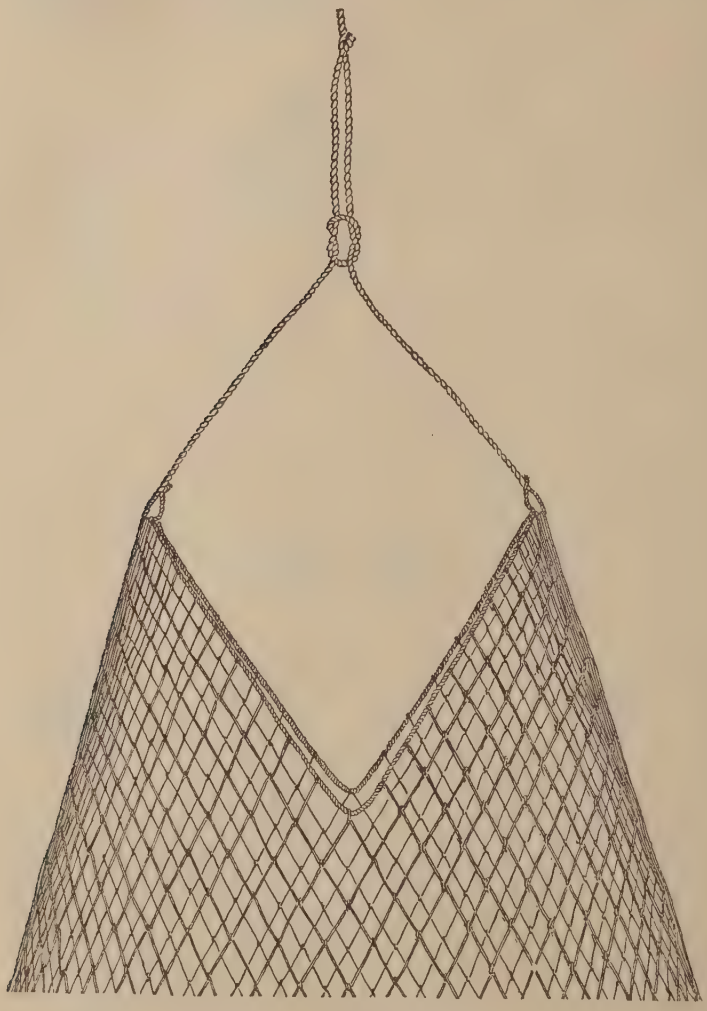

FIG. 5. Apical portion of a pangdubay showing apical V-shaped cut.

more or less directly attached to it, the apical end of this particular dala, which is composed of 72 meshes, has a vertical V-shaped cut (text fig. 5). Twenty-two meshes of the webbing

on each side of the rim of this apical cut are attached to a $\frac{1}{8}$-inch marginal cotton cord. The free ends of these marginal cords together with fourteen meshes on each side of the cut are laced to the eye-splice formed of the free ends of a $\frac{1}{2}$-inch bridle cord about 7 feet long. To the bight of the bridle cord is then attached the hauling-in line proper in the manner shown in the text figure. This opening is especially useful when removing the baited line from inside the cast net during the hauling operation.

The various kinds of deep-water cast nets in use in Manila Bay are the following:

Serial No.	Name of gear.	Kind of twine.	Size of twine; No.
1	Pangsiliniasî or panglamok	Linen	50 and 40.
2	Malimit na pamulang usô	do	50 and 40.
3	Pamulang usong talaga	do	50 and 40.
4	Pang-alañgan	do	50 and 40.
5	Malimit na panghalobaybay	do	50 and 40.
6	Panghalobaybay or pangtunsoy	do	50 and 40.
7	Panamban	do	28.
8	Pangduhay	China grass	$\frac{1}{32}$" to $\frac{1}{16}$" diam.

Serial No.	Size of stretched mesh.	Fish caught.	Price of net.	Season of operation.
	in.		*Pesos.*	
1	$\frac{1}{2}$	Young herrings	42	February to May.
2	$\frac{5}{8}$	Small herrings	30	Do.
3	$\frac{3}{4}$	do	27	Do.
4	$\frac{7}{8}$	Herrings of intermediate size	25	Do.
5	$1\frac{1}{8}$	Medium-sized deep-bodied herrings	25	June to December.
6	1	Adult herrings	20	Do.
7	$1\frac{1}{4}$	Adult sardines	20	Do.
8	$3\frac{1}{2}$	Pomfrets	15	October to December.

METHOD OF OPERATION

The fishing boats leave port for the fishing ground (different points around the Bay) early in the morning. Upon arrival at the fishing ground, they reconnoiter for schools of pelagic fishes, usually herrings. The net caster, who stations himself in the prow of the boat, making use of the wooden platform as his stage, scouts for the schooling fishes.

When such a school is sighted, he signals to the steersman to direct the boat towards it. He then prepares a net for casting. This he does by coiling the hauling-in line on the platform. Then he piles the net in folds. Grasping the folds of netting

with the right hand (in the case of a right-handed fisherman) he arranges the lower marginal edge of the cast net so as not to entangle the sinkers and at the same time make a perfect spread of the net. With his right arm he supports a portion of this edge of the net towards the right. He then spreads apart the remaining lower edge towards his left with the left hand. In this position (Plate 1, fig. 2) he waits until the boat approaches the vicinity of the school.

In the meanwhile, when the boat is about sixty feet from the school, the rowers change their oars for the paddles in order that they may approach it as close as possible with the least

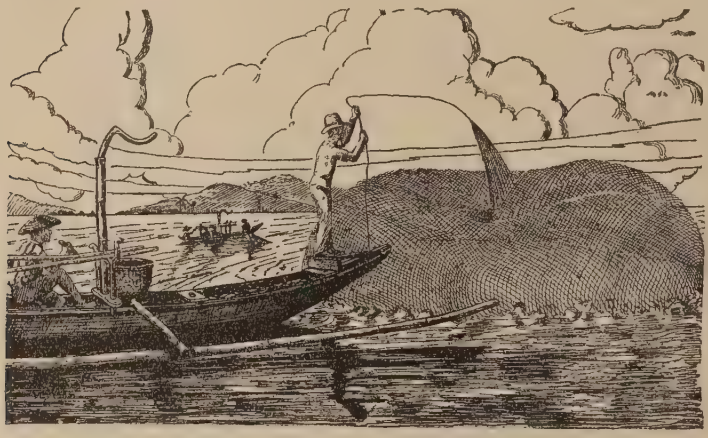

FIG. 6. The deep-water dala in operation.

disturbance of the water. No unnecessary motions and disturbances are made during this phase of the operation, as a slight movement is enough to frighten the fish away.

The caster determines the movement of the school and at the same time locates its head. The boat is then maneuvered to have the school head towards it. When within the reach of his throw, he casts the net with a deft half-swing of the body—whereby it is thrown forward, opening out gracefully in an almost perfect circle (Plate 1, fig. 3; Plate 2, fig. 2; and text fig. 6). As the weighted edge sinks, the net is dragged forward—whereupon a considerable length of the retrieving line is released. The fishes are impounded and usually gilled in the twine as the net settles.

The thrower begins to haul in the cast net by pulling or drawing on board the hauling-in line from the side of the boat (Plate 2, fig. 1). While thus being drawn, the edges come together, collapsing the side of the spread net, thus insuring to a greater extent the inclosing and the gilling of the fishes. The cast net proper, with the impounded and the gilled fishes, is hauled in (Plate 2, fig. 3). It is then hung on the hooklike pole and the fish are removed by one or more fishermen (Plate 3, figs. 1 and 2).

Th oarsmen again row the boat at full speed to locate another school of fish and the operation is repeated.

As each boat carries from seven to eight nets, successive throws are made, depending upon the abundance of the schools of such fishes. The gilled fishes are quickly removed, so that the net can be cast again.

While this description holds true with the majority of the dala, the pangduhay is operated in a somewhat different manner. In the case of this gear, the baited lines are first set at various points in the fishing ground. From time to time these lines are examined to ascertain the presence of a fair number of pomfrets. This is done by dipping the float of the line with the aid of the pamangkao (the long-handled scoop), slightly lifting the baited end with care. When a good number of such fishes have been attracted, the line is cautiously returned to its original position in the water. The dala is then thrown over the baited line impounding and gilling the pomfrets. In the hauling process, this accessory apparatus is removed through the V-shaped opening of the apical edge. Then the whole net is hove up from one side of the boat and the catch picked from its meshes.

THE CATCH

The catch, composed of small and medium-sized pelagic species that run in schools, is then placed in bamboo baskets.

The most important species caught are the sardines (*tamban* when full grown and *tamban siâ* when immature), *Harengula longiceps* Cuv. and Val.; the round-bodied herring (*tunsoy* when full grown and *siliniasi* when young), *Harengula molluccensis* Bleeker; and the deep-bodied herring (*lapad* or *halobaybay* when full grown and *bagasbas* when young), *Harengula fimbriata* Cuv. and Val. Sometimes small quantities of slipmouths (*sapsap, e-im, dalañgat,* and *hualing*), *Leiognathus* spp.,

are included in the hauls. Pomfrets (*duhay*), *Stromateus niger* (Bloch), comprise the pangduhay catches.

Although some casts are entirely fruitless, the majority of the hauls bring fairly good catches—at times, very abundant within a short period of actual fishing. These catches are disposed of at the nearest local market, as they are never iced. When extraordinarily large, however, they are taken to Umbuyan (that section of Tondo where the smoke houses are located) where they are sold wholesale to Chinese owners of smoke houses.

ILLUSTRATIONS

PLATE 1

FIG. 1. Two boats used in dala fishing in the deep water.
2. The dala caster ready for the throw.
3. The throw.

PLATE 2

FIG. 1. Hauling the net from the side of the boat.
2. Closer view of the throw.
3. The catch being hauled in.

PLATE 3

FIG. 1. The net with the catch hung on the hooklike pole.
2. Picking the gilled fish from the meshes of the net.

TEXT FIGURES

FIG. 1. The apical portion of a typical dala, showing attachment of hauling-in line.
2. Portion of weighted edge of the dala, showing the way the lead sinkers are attached.
3. A baited line, an accessory part of a pangduhay.
4. The pamangkao, a long-handled scoop.
5. Apical portion of a pangduhay showing apical V-shaped cut.
6. The deep-water dala in operation.

1

2

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PLATE 1.

1

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PLATE 2.

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PLATE 3.

THE NUTRITIVE PROTEIN VALUE OF FIVE VARIETIES OF RICE

By A. J. Hermano

Of the Bureau of Science, Manila

SIX TEXT FIGURES

Rice constitutes the chief staple food of the inhabitants of the Philippine Islands and other Oriental countries; such as, Japan, China, Siam, India, the Dutch East Indies, and the Malay Peninsula. There are many varieties and very little attention and study have been devoted to the proper variety selection with regard to the biological value of the protein content. A variety of rice with low protein content might possess more nutritive value than others with a higher protein content.

Wells, Agcaoili, and Feliciano [1] reported that scientific control of fertilizers, cultural methods, and irrigation are important factors in improving the yield and the quality of rice as to protein and carbohydrate content.

Vedder and Feliciano [2] have shown that amino-nitrogen is useless as a chemical index for beriberi-preventing rice. They proposed, however, the following indices for beriberi-preventing rice: *"Any rice having 1.77 per cent of phosphorus pentoxide plus fat, but not less than 0.4 per cent phosphorus pentoxide; or any rice having not less than 0.62 per cent phosphorus pentoxide; or any rice having not less than 0.50 per cent phosphorus pentoxide and with at least 75 per cent of the external layers of the grain remaining."*

Suzuki, Matsuyama, and Hashimoto [3] separated pure rice protein and fed it to albino rats. They demonstrated that rats on a diet containing 10 per cent of rice protein never weighed more

[1] Philip. Journ. Sci. 20 (1922) 353–361.
[2] Philip. Journ. Sci. 35 (1928) 351–389.
[3] Institute of Physical and Chemical Research 4 (1925) 1–48.

than 130 grams. Standard growth was obtained, however, by employing 15 per cent or more of rice proteins in the diet.

EXPERIMENTAL PROCEDURE

For this investigation, five varieties of rice were used. Polished samples of Sipot, Mancasar, Ramay, Binicol, and Apostol were supplied by the Bureau of Plant Industry. Each variety of rice was cooked in the same way as the Filipinos boil it for their meals. McCollum's salt mixture No. 185, purified butter fat, and Bureau of Science tikitiki extract were employed in the basal ration used for the experiments as follows: Rice, 86 grams; salt mixture No. 185, 4; butter fat, 5; and tikitiki extract, 5.

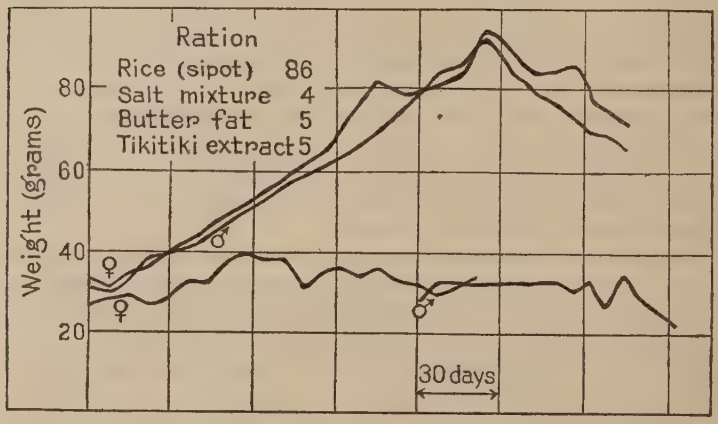

FIG. 1. Weight curves of albino rats fed on Sipot rice as the source of proteins.

The chemical analyses of the rice samples were made according to the methods of the Association of Official Agricultural Chemists.[4] The object of the analyses was to ascertain the protein contents of the different varieties of rice.

The biological study was performed on twenty-seven healthy albino rats. Eight rats were used for experiment on the Sipot variety, five for Mancasar, four for Binicol, four for Ramay,

[4] Official and Tentative Methods of Analysis. Association of Official Agricultural Chemists (1925).

and six for Apostol. The animals after weaning were subjected to diets which contained polished rice as the sole source of proteins. The diets were faulty to a certain extent because the total protein contents were below the requirement for normal growth. The proteins were not separated from the rice. Feeding experiments of separated proteins from different varieties of rice remain to be investigated.

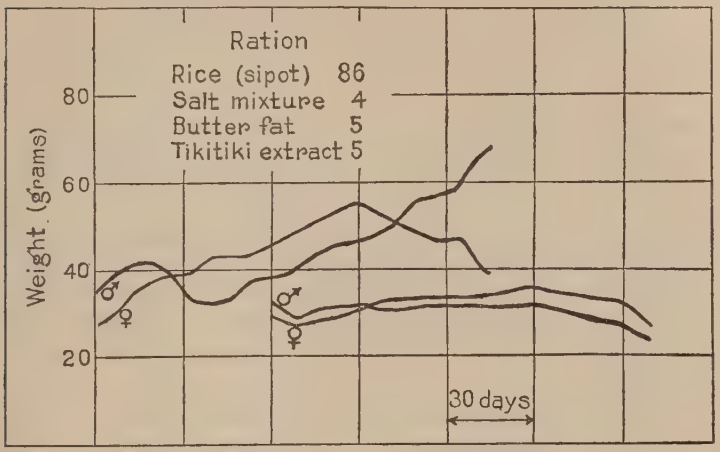

FIG. 2. Weight curves of albino rats fed on Sipot rice as the source of proteins.

RESULTS

Table 1 gives the chemical analyses of five varieties of rice, and text figs. 1 to 6 demonstrate the biological tests on albino rats.

TABLE 1.—*Results of analyses of five varieties of rice.*

Variety.	Moisture.	Protein (Nx6.25).	Ether extract.	Ash.	Carbohydrates (by difference).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Apostol	14.53	8.01	1.56	1.29	74.61
Binicol	12.75	8.32	0.84	1.54	76.55
Mancasar	11.39	7.11	0.85	0.61	80.04
Ramay	11.81	6.20	0.65	0.54	80.80
Sipot	11.22	11.54	0.61	0.73	76.90

TABLE 2.—*Nutritive value of five varieties of rice.*

Variety of rice.	Total protein.	Rats used.	Maximum body weight.	Duration of experiments.	Remarks.
	Per cent.		*g.*	*Days.*	
Apostol	8.01	6	72.0	108–204	Five of the six rats employed in the experiments did not reach 45 grams though one weighed 73.5 grams.
Binicol	8.32	4	69.0	93–133	One rat reached the weight of 69 grams and the other three were stunted.
Mancasar	7.11	5	104.3	94–153	Two of the five rats reached the weights of 82 and 104.3 grams, respectively. One rat died of respiratory disease.
Ramay	6.20	4	75.0	123–207	One rat reached a weight of 75 grams, and one died of respiratory disease.
Sipot	11.54	8	94.3	93–195	Two of the eight rats used in the experiment reached the weight of 93 and 94.3 grams, respectively. Four were stunted and one died of loss of weight.

As shown by the data in Table 1, the protein contents of the five varieties of rice were found to vary from 6.20 to 11.54 per cent.

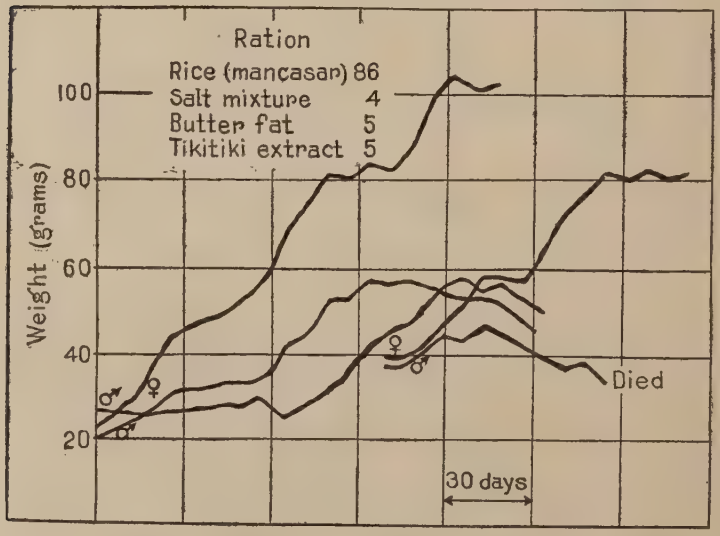

FIG. 8. Weight curves of albino rats fed on Mancasar rice as the source of proteins.

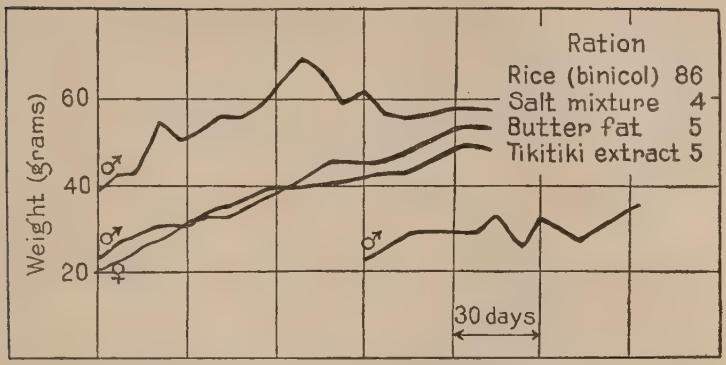

FIG. 4. Weight curves of albino rats fed on Binicol rice as the source of proteins.

The growth curves of figs. 1 to 6 indicate that rice as the sole source of protein in the diets was not adequate to meet the needs of the rats. The animals were stunted and never weighed more than 104 grams, which is an indication that the diets were deficient in protein requirement.

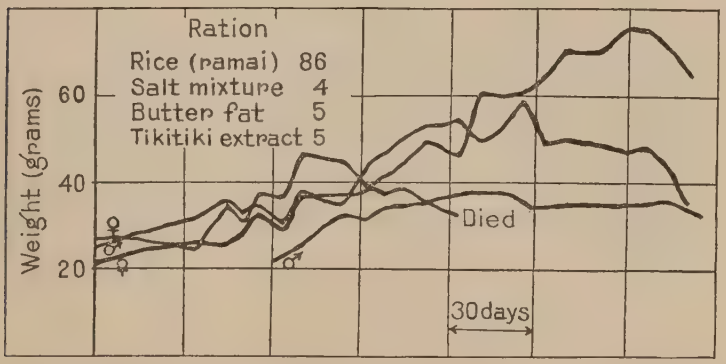

FIG. 5. Weight curves of albino rats fed on Ramay rice as the source of proteins.

The curves of figs. 1 and 2 illustrate the growth of the rats fed on diets the protein of which was derived entirely from sipot rice. The protein content in the diet was approximately 10.30 per cent.

The curves of fig. 3 show the growth of the rats fed on diets the protein of which was derived from Mancasar rice. The pro-

tein content in the diet was approximately 7.49 per cent. Although the protein content was lower than in the diets containing Sipot, Binicol, and Apostol, two rats grew larger than the other animals fed with the same amount of rice.

The chart for Ramay rice shows that Ramay has a low nutritive value.

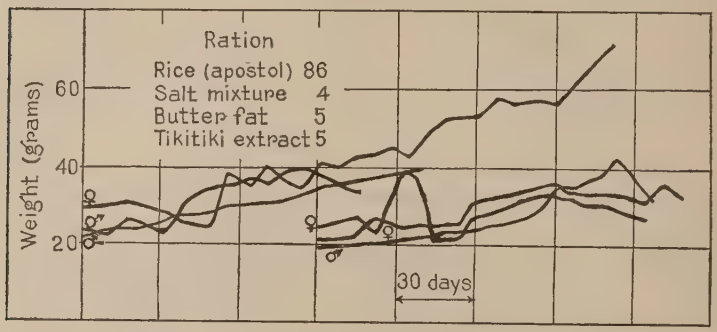

FIG. 6. Weight curves of albino rats fed on Apostol rice as the source of proteins.

SUMMARY

1. The chemical constituents and the nutritive protein value of five varieties of rice were determined.

2. Mancasar was found to contain less protein, but its nutritive value seemed to be greater than that of Apostol, Binicol, and Sipot.

3. Ramay was found to contain the least amount of protein but its nutritive value appeared to be higher than that of Apostol and Binicol.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Weight curves of albino rats fed on Sipot rice as the source of proteins.
2. Weight curves of albino rats fed on Sipot rice as the source of proteins.
3. Weight curves of albino rats fed on Mancasar rice as the source of proteins.
4. Weight curves of albino rats fed on Binicol rice as the source of proteins.
5. Weight curves of albino rats fed on Ramay rice as the source of proteins.
6. Weight curves of albino rats fed on Apostol rice as the source of proteins.

TWO FRESH-WATER SHELLS FROM THE PHILIPPINE ISLANDS

By Leopoldo A. Faustino

Chief, National Museum Division, Bureau of Science, Manila

TWO TEXT FIGURES

In the course of his investigations of the intermediate molluscan hosts of human flukes Dr. M. Tubangui, of the division of biological laboratory, Bureau of Science, encountered two fresh-water mollusks that have not been reported from the Philippine Islands. These shells were given me for identification, and I am glad to have the opportunity to report on them.

GYRAULUS PRASHADI sp. nov. Fig. 1.

Shell discoidal, small, thin, somewhat flat, transparent or translucent, whorls five, rounded, closely and obliquely striated, peri-

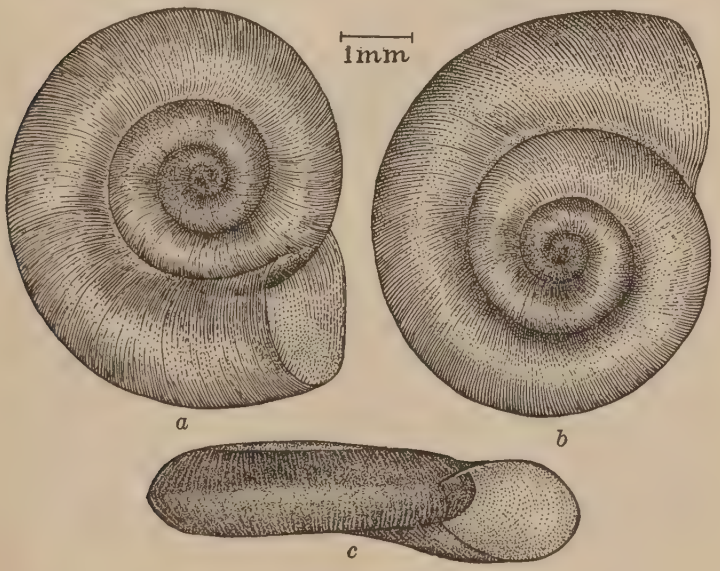

Fig. 1. *Gyraulus prashadi* sp. nov.

phery subangular, spire depressed, aperture egg-shaped. A few of the specimens are black due to the habitat.

Height, 1 millimeter; maximum diameter, 5.

Locality.—San Antonio, Zambales Province, Luzon; also in ponds around Manila and vicinity; collected by M. Tubangui.

Type.—Bureau of Science No. 14404.

This species differs from *G. convexiusculus* (Hutton), which it closely resembles, in having more whorls; and in the shape of

FIG. 2. *Hippeutis umbilicalis* (Benson.)

the mouth, which is more oviform. It differs from *G. euphraticus* Mousson in being more convex and in having the whorls more rounded, in having an egg-shaped aperture and in having the angle on the middle of the outer lip not pronounced but more like that of *G. convexiusculus* (Hutton). Doctor Prashad, of the Zoölogical Survey of India, was the one who called my attention to the fact that this species is distinct from *G. euphraticus* Mousson or *G. compressus* Hutton to which I had previously assigned the lot of specimens given me for identification. It is with pleasure that I name this species after him.

HIPPEUTIS UMBILICALIS (Benson). Fig. 2.

1837. *Planorbis umbilicalis* BENSON, Journ. As. Soc. Bengal 5: 471.
1921. *Hippeutis* (?) *umbilicalis* ANNANDALE, Rec. Indian Mus. 22: 584.

Shell discoidal, nautiliform, thin, transparent, tumid above, somewhat flat below, keeled below the middle, whorls four, last whorl large and broad, spire narrow and depressed, aperture obliquely broad, umbilicus nearly enveloped.

Height, 2 millimeters; maximum diameter, 7.

Locality.—San Antonio, Zambales Province, Luzon, collected by M. Tubangui, Bureau of Science No. 14405; Sitio Napayacan, Buguey, Cagayan Province, Luzon, Bureau of Science No. 3076.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. *Gyraulus prashadi* sp. nov.
2. *Hippeutis umbilicalis* (Benson).

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THE LIFE HISTORY OF THE HUMAN INTESTINAL FLUKE, EUPARYPHIUM ILOCANUM (GARRISON, 1908)

By Marcos A. Tubangui and Antonio M. Pasco

Of the Division of Biology and Serum Laboratory, Bureau of Science Manila

FOUR PLATES AND ONE TEXT FIGURE

INTRODUCTION

The following considerations were mainly responsible for taking up the study of the life history of *Euparyphium ilocanum* (Garrison, 1908) (= *Echinostoma ilocanum*) : to determine the mode of transmission of the parasite and to work out as accurately as possible its morphology in the adult stage from properly fixed experimental material. The first consideration was motivated by their inability, when asked, to give specific information on the prophylaxis of the worm. In addition, if its mode of transmission were known, it might be possible to explain from epidemiological observations the apparently limited geographical distribution of the parasite in man in the Philippine Islands.

As Tubangui (1931) pointed out in another paper, the available descriptions of *Euparyphium ilocanum* were based on material obtained from human beings after anthelmintic treatment and such specimens may not have represented accurately the anatomical features of the parasite. For this reason it is desirable that the morphology of the fluke be known definitely so that the identity of the trematode reported by Tubangui (1931) as *Euparyphium ilocanum* from the wild rat may be verified.

HISTORICAL

The Philippine intestinal fluke was discovered by Garrison (1908), who first found the ova of the parasite in the stools of five out of over five thousand prisoners in Bilibid Prison, Manila.

Later he recovered twenty-one specimens of the adult worm from one of the infested persons after the administration of male fern as an anthelmintic. Garrison described the fluke as *Fascioletta ilocana* under the supposition that it represented not only a new species but also a new genus of trematode. Odhner (1911), however, who received four specimens from Garrison's type material, at once recognized the systematic affinities of the worm and transferred it to the genus *Echinostoma* (Rudolphi) (=*Echinostomum*).

Following Garrison's discovery, other human cases of infestation with the parasite were reported by a number of investigators. In view of the common occurrence of echinostomes in general in the intestines of birds and of the successful development in man and other mammals of certain species of avian heterophyids, as reported by Faust and Nishigori (1926) and others, some observers began to suspect that the fluke in question might be a normal bird parasite that is only accidentally transmitted to human beings. No thought was entertained that the trematode might be harbored by a mammal other than man, as was suspected by Leiper (1911) in the case of *Euparyphium malayanum*, until Tubangui (1931) found what was apparently the same species of fluke in the intestine of the wild rat, *Mus norvegicus*. Before this, Majima (1927) in Japan had reported a human case of infestation with a related rat trematode, *Echinostoma macrorchis* Ando and Ozaki, 1923.

On the basis of material obtained from rats, Tubangui redescribed the parasite and placed it in the genus *Euparyphium* Dietz, 1909, due mainly to the presence of spines on the two body surfaces. Without taking it into account then, it is now found that the combination *Euparyphium ilocanum* is a resuscitated homonym, having already been used by León (1920) for a trematode, collected by him from a Roumanian peasant, which he thought was identical with the Philippine species. Later the same author and Ciurea (1922) rectified the error by describing the Roumanian parasite as a distinct species under the name *Euparyphium jassyense*.

GEOGRAPHICAL DISTRIBUTION

Garrison's fluke has so far been found only in the Philippines. Formerly, it was believed to be limited in its distribution to the northwestern provinces of Luzon, but its presence in wild rats in Manila, as already mentioned, indicates that it has a wider geographical range.

In man, however, as shown in the following review of recorded cases, it has been reported only from northwestern Luzon or, more accurately, from the Ilocano portion of the Philippine population. To begin with, the first five cases seen by Garrison (1908) were all natives of Ilocos Sur, so that this province is the type locality of the parasite. Willets (1911), during a parasitological survey in Cagayan Valley, found a case of trematode infection in a boy 13 years of age that was most probably due to *Euparyphium ilocanum*. Willets did not exactly state the province from which the boy came, but in his introductory remarks he said that "nearly all of the persons examined were Ilocanos who migrated into the Cagayan Valley."

Hilario and Wharton (1917) next reported five cases, all of whom came from towns in Zambales Province, the population of which includes a large number of Ilocanos. In the examination of the stools of more than five hundred students of the University of the Philippines at Los Baños, Schwartz and Tubangui (1922) found two cases, one of whom was a native of Zambales and the other of Ilocos Sur. From another set of students in the same institution Tubangui and Francisco (1925) detected four other cases, all of whom were Ilocanos. One was from Ilocos Sur, two from Zambales, and one from Pangasinan.

When this investigation on the life history of the parasite was started, surveys were made in several places in northwestern Luzon for the purpose of obtaining egg material for experimental infection of snails. In San Antonio, Zambales, of thirty adult people examined, eight harbored the parasite. In Tagudin, Ilocos Sur, where ninety-one high-school students were examined, seven positive cases were detected. On the other hand, in the adjoining Province of Abra, where forty-eight students of the Lagangilang Agricultural High School and eighteen Tinguians residing in Barrio Villaviciosa, Bangued, submitted fæcal samples for examination, not a single case was found. It would appear, therefore, that the parasite does not exist in Abra, although we are told by Dr. Zacarias de Jesus, assistant professor of veterinary parasitology in the University of the Philippines, that on one occasion he encountered the eggs of the fluke in the stools of a servant girl from that province.

METHODS

This work on the life cycle of *Euparyphium ilocanum* was part of a program to determine the molluscan intermediate hosts of the trematodes found in man and in domesticated

animals in the Philippines. To carry out this program, collections of common species of fresh-water snails were made and these were allowed to breed in small balanced aquaria under laboratory conditions. The purpose of this was, of course, to have on hand clean snails for infection experiments with known trematode larvæ (miracidia) whenever the latter became available.

The eggs of the parasite were obtained from time to time from two heavily infested cases residing in San Antonio, Zambales. The stools, after the usual preliminary screening, washing, and sedimentation processes, were placed with small amounts of ordinary tap water in shallow glass dishes and kept in improvised humidors at a room temperature of 26° to 31°C. In such an environment the majority of the ova went through their full development readily and on hatching liberated active miracidia.

The mollusk which is capable of playing the rôle of primary intermediary host was determined by placing active miracidia in the water containing the experimental snails. From time to time thereafter several individuals of the latter were crushed and examined for the presence of trematode larvæ. In the beginning large numbers of miracidia were used in infecting snails. After the discovery of the primary intermediate hosts, it was found necessary to use only a limited number, approximately in the proportion of one miracidium to one snail; otherwise many, if not all, of the snails died before the larvæ had had the chance to reach their full development, apparently as the result of heavy infestations.

The adult stage of the fluke was obtained by feeding infective larvæ (encysted metacercariæ) to clean laboratory white rats, cats, and monkeys.

After establishing the life history of the parasite by the use of aquarium-bred snails, field surveys were made in San Antonio, Zambales, for the purpose of collecting naturally infested mollusks and finding out how the worm is transmitted to human beings. The data obtained were used to check the laboratory findings.

In the study of the larval stages both fresh and stained specimens were utilized. In addition observations on the behavior of the miracidium in the prepared juices of different species of snails were made. Snail juice was prepared by triturating one or several individuals of a particular species of

snail in a small dish or mortar, with or without their shells, into a more or less compact mass and allowing the juice to separate by tilting the container. By means of a capillary pipette one or more miracidia were transferred to a slide with a minimum amount of water. After adding a drop of the snail juice a cover slip was put on and the slide examined under a microscope.

THE MOLLUSCAN INTERMEDIATE HOSTS

Primary intermediate host.—The following species of freshwater snails were tested with the miracidium of *Euparyphium ilocanum: Pila luzonica* (Reeve), *Vivipara burroughiana* Lea, *Thiara asperata* (Lamarck), *Amphipeplea quadrasi* Moellendorff, *Bulinus hungerfordianus* Nevill, *Lymnaea peregra* (Mueller), *Planorbis umbilicalis* Benson, and *Gyraulus prashadi* Faustino, 1933 (Plate 4, fig. 3).[1] Of these snails only the last species was found capable of playing the rôle of primary intermediate host. In this snail the miracidium after penetration proceeded to develop and passed through stages that are characteristic of echinostomes in general.

Secondary intermediate hosts.—The cercaria of *Euparyphium ilocanum,* like the cercariæ of other echinostomes, assumes the infective stage by undergoing a process of encystment and becoming transformed into a metacercaria. The discovery of its place of encystment was quite accidental. Due to a shortage of aquarium jars we had to place together in one of the experimental jars *Gyraulus* and *Bulinus* and in another jar *Gyraulus* and *Lymnaea* snails. The subsequent examination of these mollusks revealed the presence of rediæ, cercariæ, and occasionally encysted metacercariæ in *Gyraulus prashadi* and only encysted metacercariæ in the other kinds of snails. This observation served as a clue and was later verified experimentally by placing together in small amounts of water cercariæ and clean

[1] In the abstract of this paper contributed to the Eighth Annual Meeting of the American Society of Parasitologists and published in the Journal of Parasitology 19 (1932) 166, *Planorbis umbilicalis* was mentioned as one of the primary intermediate hosts of the parasite. This is a mistake and was due to the hasty identification of snails during their examination for the larval stages of the fluke. It also transpires that the shells which were believed to be *Planorbis compressus* represent a newly discovered species, *Gyraulus prashadi,* which is described by Dr. L. A. Faustino in a preceding paper in this issue of the Philippine Journal of Science.

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snails and watching the disappearance of the former due to their entrance into the bodies of the latter. The snails always showed the presence of encysted metacercariæ when examined the following day. It was determined by such experiments that any of the species of fresh-water snails enumerated above, including *Gyraulus prashadi,* is acceptable as a second intermediate host.

FEEDING EXPERIMENTS

As stated above, the encysted metacercaria represents the infective stage in the life cycle of the parasite in so far as the infection of the mammalian or definitive host is concerned. If fed to such a host, it will proceed to develop to the adult stage in the small intestine of the latter. Below are given briefly the results of some of the feeding experiments. The incubation period, that is, the number of days that elapsed from the time the metacercariæ were fed until the eggs of the parasite first appeared in the fæces of the experimental animals, was 7 to 10 days in fourteen rats; 12 to 16 days in two monkeys; and 15 days in one young cat. In these animals the infection was of short duration, the eggs disappearing from their fæces after one to two weeks. Whether this phenomenon is due to the fluke being naturally short-lived or to its not being well adapted to these hosts, has not been determined. In human beings the worm seems capable of staying for a number of years. Metacercariæ were also fed to two pigeons and one pup, but the results were negative.

Experiment 1.—August 2, 1932. Rats 1 and 2 fed experimental *Bulinus* snails containing encysted metacercariæ.

August 10. Rats 1 and 2 showed trematode eggs in fæces. Rat 1 was killed August 15 and from the anterior portion of its small intestine seventeen adult specimens of *Euparyphium ilocanum* were collected. Rat 2 was killed August 16 and yielded eighty-two adult flukes.

Experiment 6.—November 4. Four snails (*Pila luzonica*) collected from a fishpond in San Antonio, Zambales, Luzon, in which many individuals of *Gyraulus prashadi* harboring the cercaria of *Euparyphium ilocanum* were found, were placed in the cage of starved rats 7 and 8. The rats gnawed through the shells of the snails and ate the soft parts.

November 11. Rat 8 showed trematode eggs in fæces. Killed November 15 and yielded sixty-five adult *Euparyphium ilocanum.*

November 14. Rat 7 positive. It became negative November 21 and when it was killed no flukes were found in the intestines.

Experiment 8.—November 7. Monkey 1 was fed experimental *Pila* snails containing encysted metacercariæ.

November 16. Negative; fed again encysted metacercariæ.

November 28. Trematode ova found in fæces. Killed December 1 and yielded eleven adult *Euparyphium.*

Experiment 10.—December 14. Cat 1 given encysted metacercariæ found in experimental *Pila* snails.

December 29. Trematode eggs found in fæces. This cat was allowed to live and was positive up to January 11, 1933. The next day the fæces were negative, and when the animal was killed no flukes were found in the digestive tract.

STAGES OF THE LIFE CYCLE

THE EGG

Description.—The eggs are refractive, oval bodies with a faint tinge of yellow. Subspherical and even elongate forms were occasionally encountered, but these proved in the majority of cases to be infertile and may, therefore, be considered as abnormal. The size in microns has been given by the different observers cited above as follows: Garrison, 88.8 to 114.7 by 53.5 to 81.9; Odhner, 92 to 114 by 53 to 82; Hilario and Wharton, 88.8 to 111 by 53.6 to 74.4; Tubangui, 85.5 to 101.5 by 54 to 64.2. The present findings agree with these figures in showing the variability in the size of the ova. One hundred eggs found in the stools of four infested individuals and measured at random, gave the following results in microns: Length 83.2 to 116.4, maximum width 58.2 to 69.7, the average size being 96.1 by 63.4. The same number of eggs measured from the fæces of the experimental rats and monkeys gave results that are very close to these figures.

The eggshell (Plate 1, fig. 1) is smooth, operculated at one pole, and double-contoured in optical section. It is, however, quite transparent and uniformly thin except for a more or less distinct thickening at the pole opposite the operculum. The latter is a small concavo-convex disk with a finely serrated edge that dovetails into the margin of the shell opening. It measures 18.5 to 21 microns in diameter.

The germinal area is a globular mass, about 16.5 microns in diameter; it is usually located behind the equator of the egg towards the nonoperculated end. It stands out rather indistinctly from the numerous yolk balls that surround it by its greater opacity and grayish color. The yolk balls are filled with a brownish granular material. The germinal area and yolk balls are contained in a vitelline sac that lies so close to the interior of the eggshell that its presence in the undeveloped egg is difficult to demonstrate. When the miracidium is formed, its presence is made evident during the movements of the larva.

Development.—In freshly voided fæces the eggs are usually in the one-cell stage (Plate 1, fig. 2); rarely they are in the two-cell stage. When left in tap water at a room temperature of 26° to 31° C. after having been washed and sedimented, their development is rapid. The germinal area divides and passes through the well-known stages of metazoan embryonic development. During the morula stage the eggs present a characteristic picture: the bunch of embryonic cells occupies a central position, surrounded by a network of empty or almost empty spaces (Plate 1, fig. 3). The latter represent the impoverished yolk balls, the contents of which have been gradually used up by the developing embryo. As the miracidium begins to take form, these are crowded out gradually and oillike globules, which possibly represent excretory products, make their appearance. When the miracidium is fully formed, there are two or three large ones of these globules abutting the larvæ (Plate 1, fig. 4).

The majority of eggs were observed to attain the miracidial stage after six to fifteen days, others after a prolonged incubation period of five to six weeks. Hatching ordinarily took place three to five days after the formation of the miracidia.

THE MIRACIDIUM

Description.—The miracidium is similar in appearance to the miracidia of most trematodes. The anterior half of the body is more bulky than the posterior half and is produced anteriorly into a conical retractile papilla. The entire surface, except in the region of the papilla, is covered with long cilia, by means of which the larva is enabled to swim very actively. These cilia arise from definite epidermal cells or plates, of which there are nineteen arranged in four transverse rows (Plate 1, fig. 5). The first row contains six small cells, which are almost triangular

in shape with their apices directed anteriorly; the second row six larger cells that are rectangular with rounded corners; the third row four cells that are also rectangular but are larger than those of the second row; the fourth row three cells that are almost as large as those of the preceding row but are somewhat triangular with their apices directed posteriorly. In the living organism these epidermal cells are arranged very close together, except where two minute lateral processes, one on each side, protrude between the first two rows of cells. In extended specimens killed in hot 10 per cent formalin solution, the cells are seen to be separated from each other by nonciliated spaces.

In the preserved state, with the anterior papilla partly protruded, the miracidium measures 61.5 to 65.5 microns in length by 27.5 to 29.5 microns in maximum breadth. When alive it averages 85 by 35 microns in size. It is provided with a small sacculate gut which reaches to the tip of the papilla by means of a narrow duct. Both the gut and its papilla are conspicuous due to their refractive granular lining. No evidence was obtained to indicate the presence of a patent mouth as has been observed by Barlow (1925) in the miracidium of *Fasciolopsis buski.* Immediately behind the digestive tract on the dorsal surface is a pair of eyes that are discernible due to their inner pigmented portions. The lenses themselves are difficult to distinguish. The eyes are often so close together that the approximation of their pigmented areas gives the impression of the presence of a single X-shaped "eye spot." Ventral and a little posterior to the eyes is a pair of neurons, probably representing the central nervous system. Two flame cells are present near the equator of the body, one on each side of the median line. Each of these excretory cells is provided with a sinuous duct that leads to the exterior through a lateral opening located between the last two rows of epidermal cells. The germinal cells occupy most of the space in the posterior half of the body.

Behaviour in water.—Immediately after hatching the miracidium begins an active life in the water. Its behavior is very much like that of the miracidium of *Fasciolopsis buski,* as described by Barlow (1925). It swims here and there with a spiraling but smooth and graceful motion and with only very momentary stops until it either dies from sheer exhaustion or succeeds in penetrating into the body of an intermediate host. Newly hatched miracidia, kept in a small dish of water floated on the surface of a screened balanced aquarium at 8 to 9 o'clock

in the morning, did not live for more than seven hours. This experiment was repeated six times, and an average longevity of 6 hours 37 minutes was obtained.

Behavior in snail juices.—In view of the interesting observations recorded by Barlow on the behavior of the miracidium of *F. buski* in the expressed juice of its intermediate host, it was considered worth while to observe the behavior of the miracidium of *Euparyphium ilocanum* not only in the juice of its primary intermediate host but also in the juices of the other kinds of snails used in the preliminary infection experiments. Similarly interesting results were obtained. It was noticed that in the juices of the latter mollusks the miracidium reacted indifferently; that is, it behaved as if it were in water with the exception that it was unable to swim as fast due to the viscosity of the medium and to the presence of bits of snail tissues that interferred with its free locomotion.

In the juice of *Gyraulus prashadi,* on the other hand, its reaction was quite different. The following changes, which are possibly identical with those occurring during the act of penetration of the miracidium into the body of a snail, were seen to occur during observation periods lasting from three and a half to four hours: The larva on coming in contact with the fluid of its primary intermediary host seemed to be stimulated, as shown by its jerky and rather hurried movements. After a few moments it quieted down, moving here and there slowly and cautiously. Very soon a part of its epidermis at the anterior end began to bulge in the form of a blister. A few minutes later it remained stationary in one place, but with its cilia moving continuously and the bulging of the epidermis becoming more pronounced. Soon one of the second row of epidermal cells was cast off and carried away by its cilia which continued vibrating long after the epidermal cell was peeled off. Another cell from the same group may next be detached and be carried away by its supply of cilia. In many of the larvæ observed, however, the three remaining cells of this row, instead of being completely cast off like the first one, were merely folded back and remained attached to the third row of cells. Somewhat later the first row of cells was discarded by being pushed forward en masse. Its anterior end being then free, the larva pulled the rest of its body out of the remaining two rows of epidermal cells by means of its active contractions. It assumed a roundish to oval form, the anterior headlike papilla was retracted into the body and the germ cells appeared

more prominent. It had, therefore, the important characteristics of a young sporocyst and may for that reason be appropriately called a miracidium-sporocyst (Plate 2, fig. 1). At the end of three and a half to four hours the larvæ died in spite of attempts to prolong their existence by periodically giving fresh snail juice.

The apparently specific reaction shown by the miracidium of *Euparyphium ilocanum* in the juice of its primary molluscan host raises the question as to whether or not the miracidia of the different species of trematodes will exhibit the same specific behavior if placed in the fluids of their respective intermediary hosts. Experiments with the solution of this question in view would be worth undertaking; and, if positive results were obtained, a useful preliminary technic in the elucidation of the life cycles of flukes in general would be made available.

Penetration of the miracidium into the body of the primary intermediate host.—The mode of penetration of the miracidium into the primary intermediate host was ascertained, as was done by Barlow in the case of *F. buski,* by placing a snail and some water containing miracidia on the depression of a bacteriological "hanging drop" slide under a cover glass. Thus inclosed, the snail makes attempts to escape and in so doing enables one to view its exposed parts under the microscope.

Miracidia were observed to be overactive in the presence of their proper intermediate host. In view of its spiraling mode of locomotion, if a miracidium strikes against any solid body, the sudden impact of its arrested movement causes it to rotate on its long axis with a characteristic boring motion. If it strikes against the shell of its host or any hard object, its arrest is only momentary, the larva soon regaining its balance and darting away as fast as ever. If it happens to hit the body of the snail, it remains as if rooted to the spot for a longer or shorter period of time even after the boring motion of the body has ceased. Those striking against the foot, head, and tentacles of the snail, however, were found to be unable to go deeper into the tissues and were soon brushed off by the movements of these parts against the shell of the mollusk. Only those that happened to strike the mantle lingered longer and seemed able to penetrate deeper. From this observation it is believed that the miracidium normally establishes itself in the body of its snail host by entering the pulmonary chamber through the mantle and possibly also through the pulmonary orifice.

THE REDIÆ

No attempt was made to follow the route of migration and determine in detail the metamorphosis of the miracidium inside the body of the snail. What probably happens is that the larva, after casting off its epidermal cells during the process of penetration, transpasses the mantle wall and enters the pulmonary chamber. From there it works its way through the adjacent soft tissues until it finally reaches the digestive gland of the mollusk. The rediæ have always been found in the latter organ. They occupy a superficial position and are so loosely attached to the liver tissues that the least pressure, as when the shell of an infective snail is broken even very carefully for examination, is sufficient to set a large number of them free into the water.

Two kinds of rediæ are distinguished: namely, primary or mother rediæ and secondary or daughter rediæ, which in appearance and general structure do not differ materially from other echinostome rediæ; such as, for example, those of *Euparyphium murinum* and *Echinostoma revolutum*, as described by Tubangui (1932). No constant differences were observed to exist between the two kinds of rediæ, except that the mother rediæ (Plate 2, fig. 3) were always found to contain in their body cavities only daughter rediæ and the latter (Plate 2, fig. 4) only cercariæ.

The young rediæ (Plate 2, fig. 2); that is, those inclosing undifferentiated germ balls recently detached from the germinal epithelium of the body cavity, are very small compared with those containing mature or nearly mature rediæ or cercariæ. The body is colorless or, at most, tinted with very pale yellow; the rhabdocœle gut and locomotor appendages are prominent; and the collar is located relatively far from the anterior end. In older rediæ the body is generally colored either partly or wholly with various shades of yellow and brown, the rhabdocœle gut is relatively small and filled with a blackish material, the locomotor appendages are not very conspicuous, and the collar is pushed nearer the anterior end of the body. A mature mother redia contains from three to twenty daughter rediæ in different stages of development and a mature daughter rediæ from three to twenty-five cercariæ, also in different stages of development. Measurements taken of preserved specimens killed in hot 10 per cent formalin solution gave the following data in millimeters: Young rediæ,

length 0.175 to 0.250, maximum diameter in front of locomotor appendages 0.040 to 0.056, pharynx 0.020 to 0.025 across; older rediæ, length 0.325 to 1.15, maximum diameter 0.108 to 0.165, pharynx 0.04 to 0.06 across.

It was found difficult to determine accurately the details of the excretory system of the rediæ. The number of the flame cells seems to depend upon the age or stage of development of the larva. Their arrangement, however, is basically constant in being bilaterally symmetrical, the cells on either side being arranged into an anterior and a posterior group. In a small daughter redia, harboring three cercariæ, there were seen twelve flame cells on each side with their short and slightly tortuous ducts. The excretory pores are lateral, one on each side, immediately in front of the corresponding locomotor appendage (Plate 2, fig. 5).

THE CERCARIA

Description.—The cercaria of *Euparyphium ilocanum* (Plate 3, figs. 1 and 2) can be recognized at a glance through the microscope as that of an echinostome due to the presence of a head collar, a pair of prominent excretory collecting tubes filled with concretionlike bodies, and numerous cystogenous glands. It possesses the characters of the "Echinata" group of echinostome cercariæ proposed by Sewell (1922) and is most closely allied with that author's Cercaria Indicae XII found in *Gyraulus euphraticus* and *Indoplanorbis exustus*. The first snail, it will be noted, is congeneric with the primary molluscan host of the Philippine fluke.

The body and, to a lesser extent, the tail, like those of most cercariæ, are capable of marked extension and contraction, for which reason it is difficult to give their dimensions accurately. In free-swimming larvæ the body is pyriform and generally shorter than the tail. In preserved specimens this shape may be retained or the body may become elongate, in which case it may be even longer than the caudal appendage. Measurements based on such preserved material gave the following figures in millimeters: Body, length 0.18 to 0.30, maximum diameter (across acetabulum) 0.10 to 0.13; tail, length 0.13 to 0.35, maximum diameter (near base) 0.035 to 0.050; cephalic collar 0.075 to 0.098 across; oral sucker 0.040 to 0.062 by 0.045 to 0.070; prepharynx 0.012 to 0.020 long; pharynx 0.020 to 0.025 across; œsophagus 0.04 to 0.10 long; acetabulum 0.045 to 0.066 by 0.050 to 0.062. The oral sucker is antero-subterminal, while

the acetabulum is between the middle and last third of the body length. The œsophagus divides in front of the acetabulum into two simple cæca that extend near the posterior end of the body. Both the œsophagus and cæca are composed of single columns of cells, the shape of which, depending upon the state of contraction or relaxation of the body, may be either square or rectangular. The reniform cephalic collar is armed with fifty-one spines arranged in two alternating rows uninterrupted dorsally. These collar spines are delicately needle-shaped and are of about the same size, 8 to 10 microns in length. The body surface behind the collar is beset with numerous minute spines, directed posteriorly and arranged in transverse rows. Beneath the cuticle from immediately behind the level of the pharynx to the posterior end of the body are numerous cystogenous glands. These are transversely oval, pear-shaped or roundish bodies, measuring about 12.5 microns across and filled with a finely granular material which renders obscure the outlines of the other structures in the body. The genital anlage is represented by two masses of cells, one in front and the other behind the acetabulum, connected by a narrow strip of cells passing dorsal to the acetabulum.

The excretory system (Plate 3, fig. 3) is typical of echinostomes in general arrangement. There is a median, contractile, excretory bladder near the posterior end of the body that communicates with the outside through a posterodorsal excretory pore. A posterior collecting tube originates from the posterior portion of the bladder; a short distance behind the base of the tail, this tube divides into two branches, each of which opens outside through a lateral pore. The excretory bladder is constricted at its anterior portion and thus gives rise to a small secondary bladder, from which the two principal excretory vessels, the main lateral collecting tubes, arise. The latter follow an anterior zigzag course on each side of the body; at first they are narrow in diameter, but in front of the middle level of the acetabulum they are dilated and filled with globular refractive bodies, the presence of which render these tubes very conspicuous. Each of these tubes on reaching the region of the cephalic collar becomes smaller in caliber, bends outwards and then inwards and backwards, thus forming a loop just behind the oral sucker. The reduced tube is continued posteriorly as an ascending limb, the latter being closely applied against the outer wall of the main collecting tube up to the middle level of the acetabulum and therefore difficult to detect. Behind that level

it separates from the main tube, and on reaching the level of the excretory bladder it gives off two sets of three capillaries with their corresponding flame cells. Then it describes a small loop, takes an anterior course (descending limb), and near the region of the oral sucker it breaks up into three capillaries with their corresponding excretory cells. The total number of flame cells is fifteen pairs, the distribution of which is shown in Plate 3, fig. 3. Due to the presence of the cystogenous glands it was not possible to trace accurately the capillary connections of the remaining six pairs (4th to 9th, inclusive) of these cells.

Liberation of cercaria from its host.—In two experimental infestations it was observed that snails began to yield cercariæ in from forty-two to fifty days after exposure to the miracidia. By placing some of these infested snails singly with small amounts of tap water in small glass vials, it was further observed that the escape of the cercariæ from their hosts occurs during the day, between 11 in the morning and 5 o'clock in the afternoon. The greatest number emerged between 1 and 3 o'clock. This finding agrees with the observations of Cort (1922) and Rees (1931), who determined that in the case of other echinostome cercariæ their emergence from their snail hosts also occurs during the daytime only. It further agrees with the explanation given by Rees, who ascribes the phenomenon to an accelerated rate of development of the larvæ during the day on account of the gradual rise of temperature because of the following observation. In the Philippines during the cool days of December, it was often noticed that the appearance of the cercariæ was much delayed when vials containing infested snails were left standing near an open window. In another lot of vials placed near an incubator or in a warm chamber, the emergence of the larvæ was much accelerated.

By removing and counting the larvæ escaping into the water at one- to two-hour intervals, estimates were made of the total number of cercariæ a single infested snail could produce each day. Counts made from six snails and extended for five days gave a total daily yield of from 58 to 194 cercariæ per snail, with an average of 119. One snail had a nearly uniform daily yield of from 190 to 194, while in another one the number ranged from 72 to 186. These figures most probably are due to differences in the number of mature daughter rediæ harbored by a particular mollusk, but they are significant in demonstrating the potentially large number of infective metacercariæ that a single snail can produce daily.

Method of locomotion and duration of free existence.—The cercaria is an active swimmer, its attitude when thus engaged is similar to that of *Cercaria* "Z," as described by Rees (1931). The body is bent ventrad so that it is concavo-convex; the tail continues along the ventral surface and then bends back again just behind its root. The result is that the dorsal surface of the body is foremost with the tail lashing on the ventral surface. When the larva comes in contact with a substratum, its method of locomotion is by creeping; that is, by the alternate attachment of its suckers to the surface.

The duration of the free existence of the cercaria is brief, its active life lasting not more than ten hours under laboratory conditions. After that time it sinks to the bottom and is soon dead. Recently emerged larvæ, placed at 4 to 5 o'clock in the afternoon in small, cotton-plugged test tubes filled with water and stood under the surface of the water in a balanced aquarium, were invariably found dead the following morning.

ENCYSTED METACERCARIA

Formation.—Like other echinostome cercariæ, the cercaria of *Euparyphium ilocanum* has to undergo a process of encystment and be transformed into a metacercaria before it is able to infect a suitable vertebrate host. It is obviously for these reasons and in order to look for a favorable spot for encystment that it escapes from its redia after reaching a certain stage in its development. It has never been found to encyst inside its redia, as is the case with some of the other echinostome cercariæ. By keeping it under close observation in different kinds of environment, it was determined that it is also unable to encyst either in water or on aquatic plants and that it can only do so in the body of a second intermediate host, which is a snail. If a free-swimming cercaria comes in contact with any exposed portion of the body of such a host, it attaches itself tenaciously by means of its suckers and by the use of the same organs it works its way into the pulmonary chamber, presumably through the respiratory opening.

The process of encystment consists essentially in the shedding around its body surface of the secretion of the cystogenous glands, which on hardening forms a protective envelop for the inclosed larva or metacercaria. Simultaneously with the formation of the cyst, the tail becomes gradually detached from the body. Encystment can apparently take place on any portion of the inner wall of the mantle cavity, but the sites of predilection are the soft tissues around the pericardium and at the hinder por-

tion of the digestive tract, the wall of the pulmonary sac in those snails possessing such a structure, and the surface of the liver.

Although, as stated in an earlier section of this paper, the cercaria is able to encyst in many kinds of fresh-water snails, it was noted repeatedly in experiments conducted for the purpose of determining the second intermediary host of the parasite that the larva is especially attracted by the large ampullarid *Pila luzonica* (Plate 4, fig. 4). When large numbers of free-swimming cercariæ were placed in small beakers of water with this snail, they disappeared completely due to encystment after thirty to forty minutes; with the other kinds of snails, after one and one-half to three or more hours. This may be taken as an indication that *Pila luzonica* is probably the normal second intermediate host of the parasite. As far as the transmission of the fluke to human beings is concerned, it is believed that such is very probably the case; for, as will be shown later, this particular snail is often eaten raw by many people in the endemic places.

With reference to the presence of cysts in *Gyraulus prashadi*, which also serves as a primary intermediate host, some explanation seems necessary. The fact that cysts were found only in those individuals of this snail which harbored rediæ and cercariæ is taken as an indication that the origin of these cysts was not from cercariæ that had already escaped into the water. What probably happens is that, due to certain obstacles, the exit of some of the cercariæ, from either their rediæ or the mantle cavity of the snail, is delayed to such an extent that when the proper time for encystment arrives the cercariæ still find themselves within the body of their first intermediate host.

Description.—The cyst (Plate 1, fig. 6) is a globular or slightly oval structure, inside which the metacercaria lies bent on itself. It is double-walled; the outer wall is thin and membranous, measuring 120 to 133 by 108.2 to 129 microns; the inner cyst wall is 2 to 3 microns thick and measures 116 to 129 by 105 to 125 microns. The space between the two walls seems to be filled with a fluid substance. In view of the presence of only one variety of cystogenous glands in the cercaria, it is believed that only the inner cyst wall is produced by the parasite, the outer wall being probably of host origin.

Resistance and longevity of cysts.—The cysts are quite frail, the outer cyst wall being readily broken even by the slightest mechanical pressure. The inner cyst wall is more resistant and less easily penetrated by a chemical agent, like a 4 per cent solu-

tion of acetic acid. The inclosed metacercaria is quickly destroyed by drying and by exposure to the rays of the sun. In the body of a snail the cysts can remain alive and infective for about a week after their formation; after that time the inclosed metacercariæ are usually dead or, if they are still alive, their viability is very much reduced. A cyst containing a dead metacercaria is recognized by its greater transparency due to the disintegration of the larva and, of course, by the absence of any movement on the part of the metacercaria.

THE ADULT

The adult specimens collected from experimental animals represent the parasite in different stages of its growth. Although they exhibit wide variations in the dimensions, not only of the body but also of the various internal organs, they possess the general characteristics of *Euparyphium ilocanum* (Plate 4, fig. 1) and are provided, as pointed out in an earlier paper (Tubangui, 1931), with fifty-one collar spines.

In view of the available descriptions by different writers, it will not be necessary to take up in detail the morphology of the adult worm. The smallest gravid specimens, 2.5 millimeters in length by 0.6 millimeter in maximum breadth, were collected from a white rat and were approximately one week old; the largest, 6.5 by 1.2 millimeters in size, were also from a laboratory rat and were about two weeks old. The cuticle is covered with numerous plaquelike spines arranged in alternating transverse rows; these spines extend on the ventral surface from the anterior end immediately behind the head collar to the posterior end of the second testis or slightly behind that level and on the dorsal surface from behind the collar to the level of the acetabulum. The kidney-shaped cephalic collar measures 0.11 to 0.15 by 0.22 to 0.34 millimeter and is armed with fifty-one spines arranged in two alternating rows uninterrupted dorsally (Plate 4, fig. 2). The five corner spines on each side of the collar are sometimes bunched together and measure 20.8 to 41.5 microns in length by 8.3 to 12.5 microns in maximum diameter; the lateral spines, of which there are thirteen to fourteen on each side, measure 25 to 45.7 by 10 to 12.5 microns; the dorsal spines, thirteen to fifteen in number, are 20.5 to 37.5 by 8.3 to 12.5 microns in size. The dimensions in millimeters of the different internal organs are as follows: Oral sucker 0.10 to 0.16 by 0.10 to 0.17; prepharynx 0.01 to 0.03 (length); pharynx 0.10 to 0.16 by 0.09 to 0.14; œsophagus 0.09 to 0.18 (length); aceta-

bulum 0.30 to 0.50 by 0.34 to 0.58; cirrus sac 0.26 to 0.54 by 0.13 to 0.27; ovary 0.10 to 0.32 by 0.13 to 0.37; shell gland 0.13 to 0.35 by 0.22 to 0.64; anterior testis 0.20 to 0.68 by 0.19 to 0.60; posterior testis 0.26 to 0.80 by 0.19 to 050. The ovary is usually transversely compressed; rarely it is globular. The testes in very young adults may possess smooth margins, but more often each organ is constricted either at the equator of its length or in several places so as to be divided more or less distinctly into lobes.

EPIDEMIOLOGICAL OBSERVATIONS

Infective agents.—Although no actual tests were made on man, it can be taken for granted from the results of the feeding experiments that human infestations with *Euparyphium ilocanum* are brought about by the consumption of raw or insufficiently cooked snails harboring the living encysted metacercariæ of the parasite. This conclusion is supported by epidemiological data gathered in Zambales and Ilocos Sur, where it was found that all those who were detected by fæcal examination to harbor the adult worm were addicted to the use of raw snails as food. Inquiries made in many places in the Philippines disclosed the fact that this habit exists almost exclusively in the northwestern provinces of Luzon; that is, among the Ilocanos. This explains the limited geographical distribution of the parasite in the human family. The favorite snail is the ampullarid *Pila luzonica* ("cuhol" in Tagalog and "bisocol" in Ilocano), in which, as already stated, the cercaria of the fluke readily encysts. It is said that many people, while at work in the fields or engaged in catching fish, often appease their hunger by eating this snail straight from the shell. In some houses it is consumed in much the same way, while in others it is served also uncooked but with salt or "bagoong" and with or without vinegar or lemon.

Another snail which is also sometimes used as food is the so-called crow snail, *Amphipeplea quadrasi* ("susung awak" in Pampango and "birabid" in Ilocano). This is said to be poisonous if not properly prepared, and for this reason many people prefer to cook it before serving it on the table. A few, however, eat it either raw or only half-cooked so that it may also be involved in the transmission of the parasite to human beings.

Foci of infection.—It was observed during the several trips made to the endemic places that, although both the primary

and secondary intermediate hosts of the parasite are widely distributed, there are certain isolated spots where the infection prevails and which serve as propagating centers. These foci are located near river beds where there are small but permanent collections of water for snails to live in throughout the year. A typical example is a place called Caarusipan in Barrio San Miguel, San Antonio, Zambales, where the incidence of infestation among the adult members of the population was found very high. The explanation for this is in the presence of two small fish ponds, each of which is near a house, in which the adult occupants harbor the adult parasite. Besides the mudfish (*Ophiocephalus striatus*), different kinds of snails, including *Pila luzonica* and several species of planorbids, breed in these ponds. In the absence of proper sanitary facilities and practices, one does not have to stretch the imagination to account for the high percentage of mollusks found infected with the larval stages of the fluke. It was learned that the people in Caarusipan and in the neighboring places derive their supply of snails from these two ponds.

PROPHYLAXIS

With its mode of development known, as shown graphically in text fig. 1, the prevention of the parasite appears simple and consists in breaking any one of the links of the life cycle. This may be accomplished by adopting one or several of the measures that are generally recommended in the control of other trematode

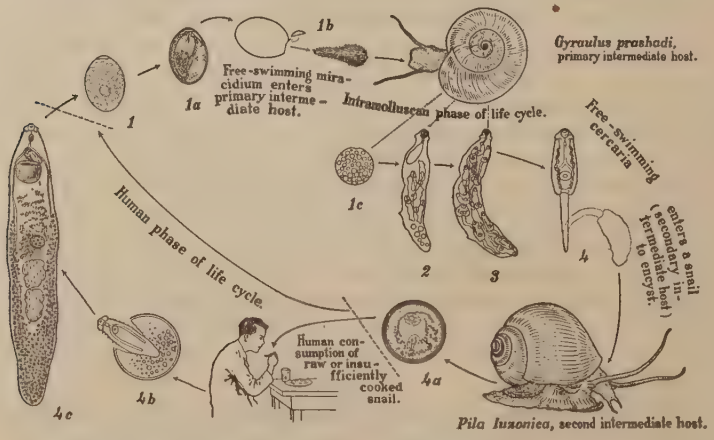

FIG. 1. Diagram of the life cycle of Euparyphium ilocanum (Garrison, 1908).

infestations; such as, the proper disposal of fæcal material, the destruction of intermediate hosts by drainage or by poisoning, the treatment on a large scale of infected individuals, the destruction of reservoir hosts, etc. Some of these measures, however, are either difficult and expensive to apply or prejudicial to the economic interests of the people. What to us appears to be the easiest and most practical mode of approach is to educate the people to dispose of their fæces properly in order to prevent the infestations of snails and, above all, to avoid the use of raw or insufficiently cooked snails as food. In these simple measures we are confident the people will gladly coöperate, if they are told of the dangers that they can avoid.

ACKNOWLEDGMENTS

In conclusion we wish to record our gratitude to those who directly or indirectly have helped us in this work. We wish especially to thank Dr. William H. Brown, director of the Bureau of Science, who supported the undertaking and enabled us to bring it to an early finish. We are indebted to Dr. L. A. Faustino, of the Bureau of Science, for the identification of snail hosts. To Mr. Simplicio Garcia, formerly of the Bureau of Science, we wish to express our appreciation for his technical assistance. To Messrs. Enrique Magsaysay and Pedro Madarang our thanks are due for various courtesies extended to us during our survey work in San Antonio, Zambales.

SUMMARY

The life history of the intestinal fluke *Euparyphium ilocanum* (Garrison) has been determined and found to conform to the echinostome type of development.

The following stages of the life cycle are described: Egg, miracidium, miracidium-sporocyst, mother redia, daughter redia, cercaria, encysted metacercaria, and adult.

Two molluscan intermediate hosts are involved; namely, a primary intermediate host, in which the parthenitæ develop, and a secondary intermediate host, in which the cercaria encysts and is transformed into an infective metacercaria.

The snail found capable of playing the rôle of primary intermediate host is a small fresh-water planorbid, *Gyraulus prashadi* Faustino.

Apparently any of the common Philippine fresh-water snails, including the primary intermediate host itself, is acceptable as a secondary intermediate host.

The eggs, which are in the one-cell stage in freshly passed stools, attain the miracidial stage in water at a room temperature of 26° to 31°C. after six to fifteen or more days. They hatch three to five days after the formation of the miracidium.

The cercariæ begin to escape from experimentally infected snails forty-two to fifty days after the exposure of the latter to miracidia.

The adult stage was obtained by feeding encysted metacercariæ derived from experimentally and naturally infected secondary intermediate hosts (mostly *Pila luzonica*) to clean laboratory white rats, a cat, and two monkeys. Feeding experiments with young pigeons and a pup yielded negative results.

The adult fluke as well as its cercarial and metacercarial stages are characterized by the presence of fifty-one collar spines arranged in two alternating rows uninterrupted dorsally.

From the results of the feeding experiments and the nature of the epidemiological data collected, it is concluded that human infestations are brought about by the consumption of raw or insufficiently cooked snails harboring the living encysted metacercaria of the parasite.

The limited geographical distribution of the fluke is explained by the observation that the habit of using raw snails as food is practiced almost exclusively by some of the people in northwestern Luzon.

The snail most commonly eaten is the ampullarid *Pila luzonica;* hence, this mollusk is considered as the most important secondary intermediate host in the transmission of the parasite to human beings.

The infection originates from certain foci near river beds, where there are more or less permanent collections of water for snails to live in throughout the year.

The prophylactic measures recommended consist in educating the people to dispose of their fæces properly and to avoid the use of raw or insufficiently cooked snails as food.

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ILLUSTRATIONS

[Drawings by A. Gonzales]

ABBREVIATIONS USED

ac, acetabulum.
av, ascending excretory vessel.
cc, cephalic collar.
cg, cystogenous gland.
cs, cirrus sac.
cv, caudal excretory vessel.
dv, descending excretory vessel.
eb, excretory bladder.
ep, excretory pore.
fc, flame cell.
ga, genital anlage.
gp, genital pore.
int, intestinal cæcum.
mt, main lateral excretory collecting tube.
oe, œsophagus.
ov, ovary.
ph, pharynx.
sg, shell gland.
t, testis.
ut, uterus.
vg, vitelline gland.

PLATE 1

FIG. 1. Outline of the egg shell with the operculum open.
2. Freshly passed egg, in one-cell stage, showing yolk balls and germinal area.
3. Early morula stage showing peripheral arrangement of almost empty yolk sacs.
4. Egg-miracidium, the latter fully developed.
5. Diagram of miracidium showing epidermal plates and lateral processes.
6. Encysted metacercaria.

PLATE 2

FIG. 1. Miracidium-sporocyst as seen in snail-juice culture.
2. Young mother redia with undifferentiated germ balls, ventral view.
3. Mature mother redia with young daughter rediæ, lateral view.
4. Mature daughter redia filled with cercariæ, lateral view.
5. Daughter redia showing excretory system, ventral view.

PLATE 3

FIG. 1. A mature cercaria after fixation in hot 10 per cent formalin solution showing relative lengths of body and tail, lateral view.
2. Mature cercaria, showing details of anatomy, ventral view.
3. Excretory system of cercaria, ventral view.

PLATE 4

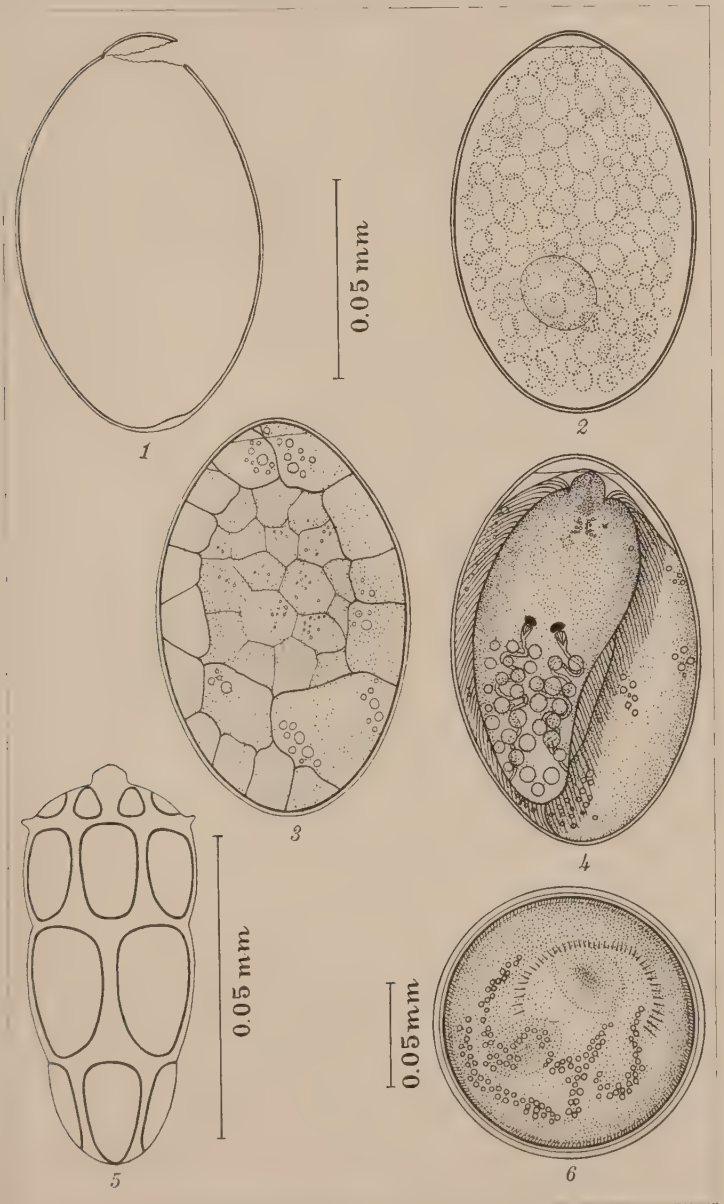
FIG. 1. Anatomy of adult worm, ventral view.
2. Cephalic collar showing number and arrangement of collar spines, ventral view.

FIG. 3. Shells of *Gyraulus prashadi* Faustino, the primary intermediate host of *Euparyphium ilocanum*, dorsal and ventral views.

4. Shell and operculum of *Pila luzonica*, one of the secondary intermediate hosts of the fluke concerned in the transmission of the parasite to human beings; ventral view.

TEXT FIGURE

FIG. 1. Diagram of the life cycle of *Euparyphium ilocanum*. *1*, Freshly passed egg; *1a*, egg with fully developed miracidium; *1b*, miracidium escaping from egg shell; *1c*, miracidium-sporocyst; *2*, mature mother redia; *3*, mature daughter redia; *4*, cercaria; *4a*, encysted metacercaria; *4b*, ruptured cyst liberating metacercaria; *4c*, adult.



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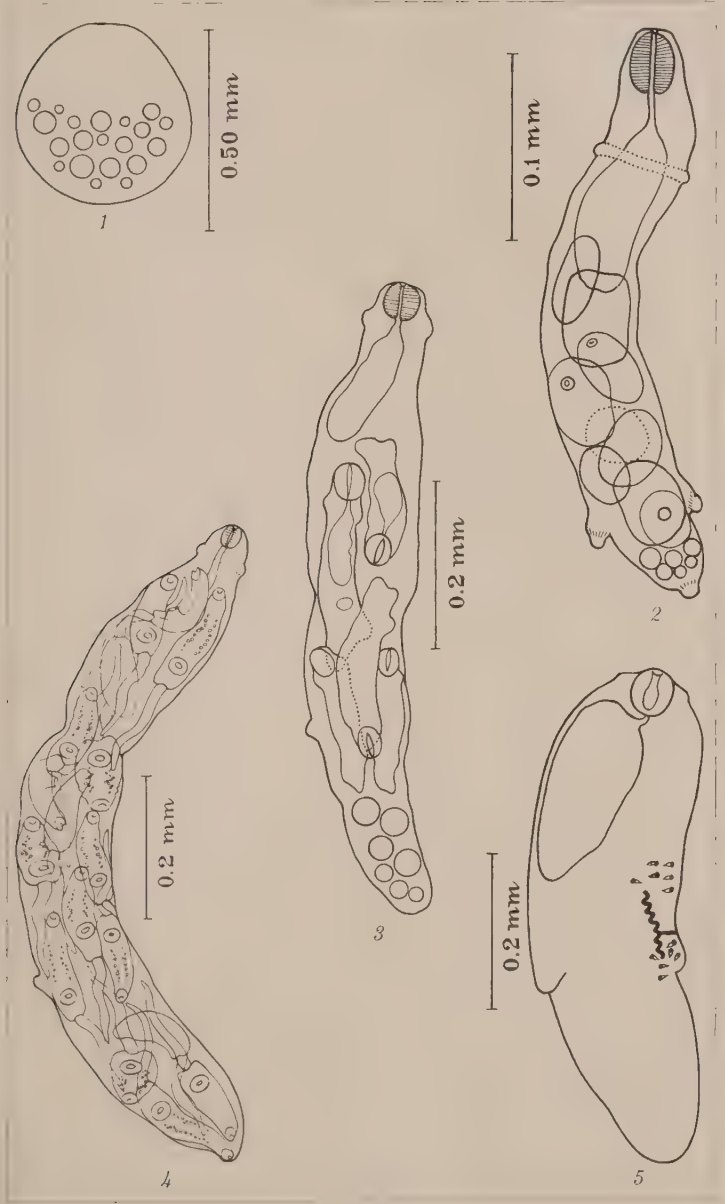

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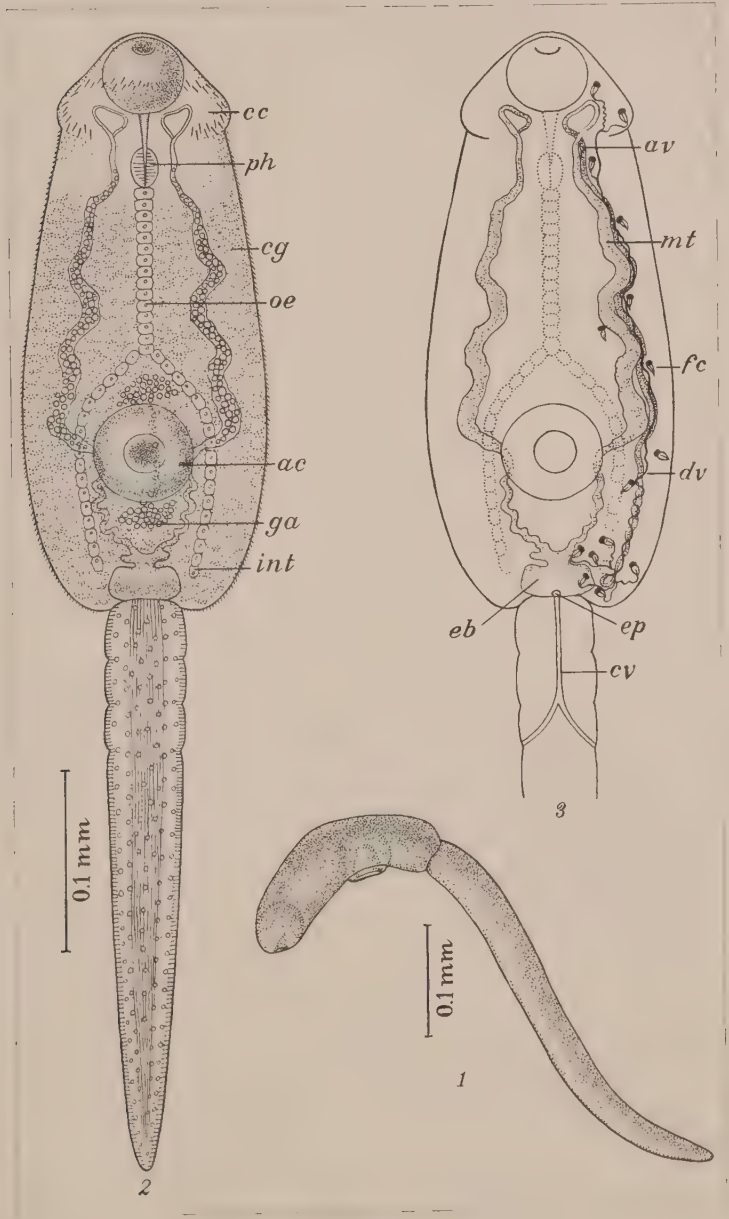

PLATE 3.

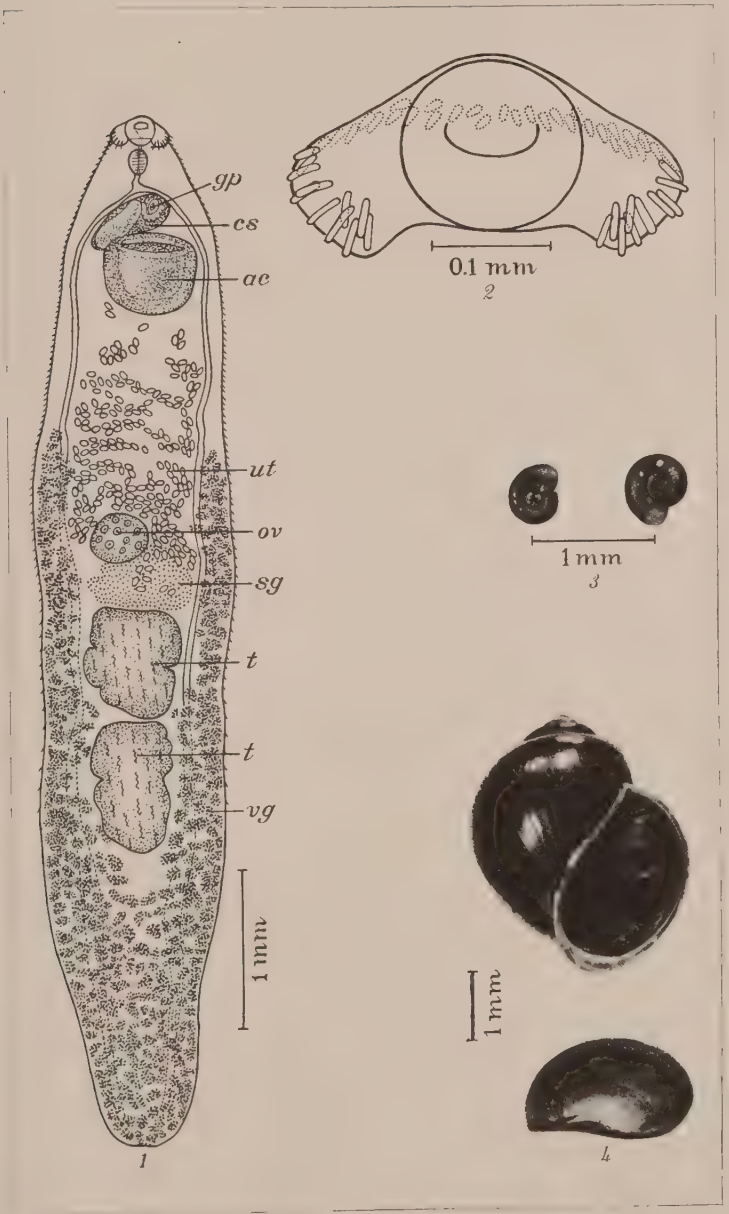

PLATE 4.

NEMATODES IN THE COLLECTION OF THE PHILIPPINE BUREAU OF SCIENCE, I: OXYUROIDEA

By Marcos A. Tubangui and Rita Villaamil

Of the Division of Biology and Serum Laboratory, Bureau of Science Manila

THREE PLATES

The nematodes infesting Philippine vertebrates, with the exception of those found in domesticated animals, have received very little attention. In the available literature there are only two papers dealing with this group of the Philippine fauna, one by Wehr (1930) on four species of bird parasites and the other by Chu (1931) on two species collected from flying lemurs.

There is at our disposal a collection of roundworms which we intend to describe from time to time, the present constituting the first of the series and relating to three members of the superfamily Oxyuroidea. The large majority of the parasites were obtained from birds by Mr. A. Duyag, to whom we wish to express our appreciation.

SUBULURA CYNOMOLGI sp. nov. Plate 1, figs. 1 to 6.

This seems to be a common parasite of the Philippine macaque, being represented in the collection by several bottles each containing numerous specimens collected from individual hosts that were brought to Manila from the neighboring provinces. According to Yorke and Maplestone (1926), Stiles and Hassall (1929), Cameron (1930), and Lee (1930), the genus *Subulura* Molin includes seven members that have been reported from primates in various parts of the world. The Philippine parasite differs from these known forms in several important characters. It appears to be most closely allied to *S. malayensis* Lee, 1930, a parasite of two species of monkeys in the Malay Archipelago, from which it may be distinguished by its larger size, proportionately shorter œsophagus, smaller gubernaculum, and longer and subequal spicules. They both possess ten pairs of caudal papillæ, but the arrangement of these is different in the two forms. In *S. malayensis* there are three pairs of preanal papillæ, one adanal and six postanal; while in *S. cynomolgi*

three pairs are also preanal, but two pairs are adanal and only five postanal.

Specific diagnosis.—Subulura: Body tapers towards both extremities, ending posteriorly in a pointed tail. Posterior end of male curved, that of female almost straight. Cuticle finely striated transversely. Mouth opening small, terminal, bounded by three simple lips. On each side of the mouth is a row of three roundish papillæ, arranged in such a way that each lip is provided with two of them. The mouth leads into a prominent cavity with a chitinous wall and measuring 0.08 to 0.10 by 0.06 to 0.08 millimeter. Arising from the floor of this cavity are three triangular teeth with their pointed ends directed anteriorly. Œsophagus long with a characteristic posterior bulb provided with a valvular apparatus. Cervical papillæ absent.

Male: Length 18 to 27.7, maximum diameter 0.36 to 0.48 millimeters. Œsophagus 2.15 to 2.37 millimeters in total length by 0.26 to 0.32 millimeter across the bulb. Nerve ring 0.36 to 0.40 and excretory pore 0.60 millimeter, respectively, from anterior end. Genital sucker oval, 0.22 to 0.24 millimeter long, supplied with radiating muscles. The sucker and the cloacal opening are 0.96 and 0.28 to 0.33 millimeter, respectively, from the tip of the tail. There are ten pairs of caudal papillæ, three of which are preanal, two adanal, and five postanal. Of these papillæ only the first adanal pair and the third postanal pair are decidedly lateral, the rest being more or less ventrally located. The two filiform spicules are subequal, the right spicule being 2.16 to 2.22 and the left 2.24 to 2.53 millimeters in length; both are 0.040 to 0.056 millimeter wide at their proximal ends and 0.02 millimeter near their distal extremities. The gubernaculum is triangular, measuring 0.14 to 0.16 by 0.058 to 0.060 millimeter.

Female: Length 22 to 30 millimeters, maximum diameter 0.48 to 0.66. Œsophagus 2.30 to 3.13 millimeters in length by 0.28 to 0.36 millimeter across the bulb. The nerve ring is 0.40 to 0.60 and the excretory pore 0.64 to 0.66 millimeter, from the anterior end. Vulva fairly prominent, located in front of the middle of the body length or 9 to 11.5 millimeters from the anterior end. Anus 1.15 to 1.50 millimeters from tip of tail. The ova found in the distal portions of the two opposed uteri are oval, thin-shelled, embryonated, 64.5 to 77.4 by 38.7 to 47.3 microns in size.

Host.—Philippine monkey (*Cynomolgus philippinensis*).

Location.—Large intestine.

Localities.—Novaliches, Rizal Province (type locality), and Los Baños, Laguna Province, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection, No. 402.

SPIRONOURA DUYAGI sp. nov. Plate 2, figs. 1 to 5.

The genus *Spironoura* Leidy, 1856, according to Yorke and Maplestone, includes fifteen species of roundworms parasitic in tortoises, snakes, and fishes. To these should be added *S. onama* described by Karve (1927) from a Burmese tortoise (*Testudo emys*). By referring to the key of Baylis and Daubney (1922) to the species infesting tortoises, which is based on the number of caudal papillæ and on the presence or absence of preanal suckerlike organs in the male, it will be seen that the Philippine parasite needs only be compared with *S. siamense* (Baylis, 1920), *S. affine* [=*Falcaustra chapini* (Boulenger, 1923)], and *S. onama*. It differs from the latter two species in being decidedly larger in dimensions, not only of the body but also of the different organs, in possessing more than one genital sucker, and in the arrangement of the caudal papillæ in the male. It is distinguished from *S. siamense* by its generally smaller size, the presence of two or three genital suckers in the male, the arrangement of the male caudal papillæ, and the absence of a pair of caudal papillæ in the female. With reference to the male genital suckerlike organs it is seen that *S. duyagi* is intermediate between *S. affine* and *S. onama* on the one hand and *S. siamense* on the other.

Specific diagnosis.—Spironoura: Body tapering towards both extremities, rounded anteriorly, conspicuously pointed posteriorly; posterior end of male curved. Cuticle transversely striated. Head not separated from the rest of the body by a distinct neck. Mouth opening triangular, bounded by three simple lips, one dorsal and two submedian, each provided with two stalks of papillæ, each of which branches into an outer and an inner process. The mouth leads into a short buccal cavity supported by a cuticular ring and measuring 0.03 to 0.04 by 0.08 to 0.09 millimeter, and then to a pharynx 0.05 to 0.07 by 0.09 to 0.12 millimeter in size. The œsophagus is divisible into three parts, according to its varying degree of chitinization, the middle of which is dark brown because it is the most heavily chitinized. The œsophagus ends in an hourglass-shaped bulb containing a valvular apparatus. This principal bulb is preceded

and separated by a constriction from a secondary bulb, the two constituting the posterior portion of the œsophagus.

Male: Length 11.5 to 13, maximum diameter 0.40 to 0.72 millimeters. Œsophagus 1.75 to 1.82 millimeters in total length; its anterior portion measures 0.69 to 0.71 by 0.13, the middle portion 0.50 to 0.56 by 0.15, and the posterior portion 0.43 to 0.47 by 0.26 to 0.27 millimeter. Nerve ring, cervical papillæ, and excretory pore 0.32 to 0.34, 0.86 to 0.88, and 1.24 to 1.26 millimeters, respectively, from the anterior end. Caudal alæ absent. Musculature of posterior end well developed. There is a series of two or three (generally two) genital sucker-like organs on the ventral surface, the most posterior of which is about 1 millimeter from the cloacal opening. These suckers are oval and about 0.22 millimeter long. The tail is short, sharply pointed, curved, and 0.47 to 0.50 millimeter long. There is a single median papilla immediately in front of the cloacal opening and ten paired ones, of which five pairs are preanal and five pairs postanal. Of the paired papillæ, the second and third postanal pairs are more or less lateral, and the rest are decidedly ventral in position. The two spicules are equal, sickle-shaped, 0.75 to 0.90 by 0.060 to 0.063 millimeter in size. They possess transversely striated walls and are inclosed in membranous sheaths up to near their pointed extremities. The poorly chitinized gubernaculum is triangular with the apex pointing towards the cloacal opening and measures 0.16 to 0.19 by 0.09 to 0.10 millimeter.

Female: Length 13 to 15, maximum diameter 0.78 to 1.0 millimeters. Œsophagus 1.82 to 1.88 millimeters in total length; its anterior portion measures 0.69 to 0.71 by 0.15, its middle portion 0.56 to 0.61 by 0.15 to 0.17, and its posterior portion 0.43 to 0.47 by 0.26 to 0.30 millimeter. The distances from the anterior end to the nerve ring, cervical papillæ, and excretory pore are 0.30 to 0.31, 0.94 to 0.96, and 1.18 to 1.26 millimeters, respectively. The vulva is 4.5 to 5.6 and the anus 0.88 to 0.97 millimeters from the tip of the tail. Caudal papillæ absent. The eggs found in the distal portions of the two opposed uteri are oval, non-embryonated, 130 to 150 by 100 to 110 microns in size.

Host.—*Cyclemis amboinensis* (Daudin).

Location.—Cæcum.

Locality.—Novaliches, Rizal Province, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection, No. 418.

CISSOPHYLLUS LEYTENSIS sp. nov. Plate 3, figs. 1 to 5.

According to Yorke and Maplestone, the genus *Cissophyllus* Railliet and Henry, 1912, is represented by *C. laverani,* the genotype, and possibly by two other species, all of which are parasites of turtles. The present form was collected on two occasions from the large intestine of the herbivorous lizard, *Hydrosaurus pustulosus* (Eschscholtz). It is smaller than *C. laverani* and its œsophagus is shorter. The spicules and gubernaculum of the male are also much smaller and the genital papillæ number nine to ten pairs, of which five to six pairs are preanal and only four pairs postanal. In the genotype there are eleven pairs of these papillæ, of which six pairs are preanal and five pairs postanal.

Specific diagnosis.—Cissophyllus: Body elongate, stout, slightly tapering towards both extremities. The posterior end is curved ventrally in the male, nearly straight in the female; in both sexes it terminates abruptly in a short tail, that of the male being more conical. The cuticle is transversely striated. The mouth is elongate dorsoventrally and is guarded by three lips. The dorsal lip is smaller but is provided with a powerful three-jointed structure ending distally in a three-lobed tooth. The subventral lips are larger, each reënforced externally by a chitinous framework of four compartments and armed with a series of closely set, pointed, lamellar processes directed towards the median line. The mouth is surrounded by six papillæ, which in position and appearance are similar to those of the type species of the genus. It leads to a buccal cavity bounded by a chitinous wall and measuring 0.13 to 0.14 by 0.12 to 0.13 millimeter. The œsophagus is long and divided into three parts according to its varying degree of chitinization. The anterior part appears poorly chitinized and is light in color; the middle part is heavily chitinized and is dark brown; the posterior part, which ends in a distinct bulb with a valvular apparatus, is intermediate in color and in degree of chitinization between the first two. Cervical papillæ are generally lacking; in rare cases a minute unpaired papilla is seen on one side in front of the excretory pore.

Male: Length 13 to 21.7, maximum diameter 0.98 to 1.50 millimeters. Œsophagus 1.93 to 2.20 millimeters in total length;

its anterior part is 0.66 to 0.74 millimeter long by 0.12 to 0.16 millimeter wide, its middle part 0.64 to 0.80 by 0.16 to 0.17, and its posterior part 0.67 to 0.73 by 0.30 to 0.40. The nerve ring and excretory pore are 0.48 to 0.52 and 1.3 to 1.5 millimeters, respectively, from the anterior end.

A definite genital sucker is absent, but the musculature of the posterior end is well developed. There are nine to ten pairs of sessile caudal papillæ, of which five to six pairs are preanal and ventral, one pair adanal and lateral, and three pairs postanal and lateral. The last three pairs of preanal papillæ are more or less bunched together, while the first pair is sometimes absent. The two spicules are similar, slightly bent in lateral view and measuring 0.56 to 0.90 by 0.05 to 0.09 millimeter. The gubernaculum is poorly chitinized, triangular, the apex directed posteriorly and ending in two short processes; it measures 0.14 to 0.20 by 0.04 to 0.06 millimeter. The cloacal opening is 0.21 to 0.30 millimeter from the tip of the tail.

Female: Length 17 to 23.5, maximum diameter 1.10 to 1.60 millimeters. Total length of œsophagus 1.96 to 2.30 millimeters, anterior portion 0.64 to 0.72 by 0.12 to 0.14, middle portion 0.63 to 0.84 by 0.16 to 0.17, posterior portion 0.66 to 0.78 by 0.30 to 0.42. Nerve ring and excretory pore 0.56 to 0.60 and 1.60 to 1.85 millimeters, respectively, from anterior end. Vulva behind middle of body; it is 4 to 6.7 millimeters and the anus 0.26 to 0.50 millimeter from the tip of the tail. The two uteri are opposed; the vagina is fairly long and is directed anteriorly from the vulva. Eggs found in the distal portions of the uteri and in the vagina are thin-shelled, non-embryonated, and measure 108 to 129 by 63.5 to 72.8 microns.

Host.—*Hydrosaurus pustulosus* (Eschscholtz).

Location.—Large intestine.

Locality.—Palo, Leyte (type locality), and Novaliches, Rizal Province. Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection, No. 425.

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ILLUSTRATIONS

[Drawings by A. Gonzales]

ABBREVIATIONS USED

an, anal opening.
ap, accessory piece (gubernaculum).
co, cloacal opening.
cp, cervical papilla.
cr, cuticular ring.
dt, dorsal tooth.
ep, excretory pore.
gp, genital papilla.
gs, genital sucker.
ll, lamellar processes.
mo, mouth.
mp, mouth papilla.
mpi, inner mouth papilla.
mpo, outer mouth papilla.
nr, nerve ring.
oe, œsophagus.
ph, pharynx.
sp, spicule.

PLATE 1. SUBULURA CYNOMOLGI SP. NOV.

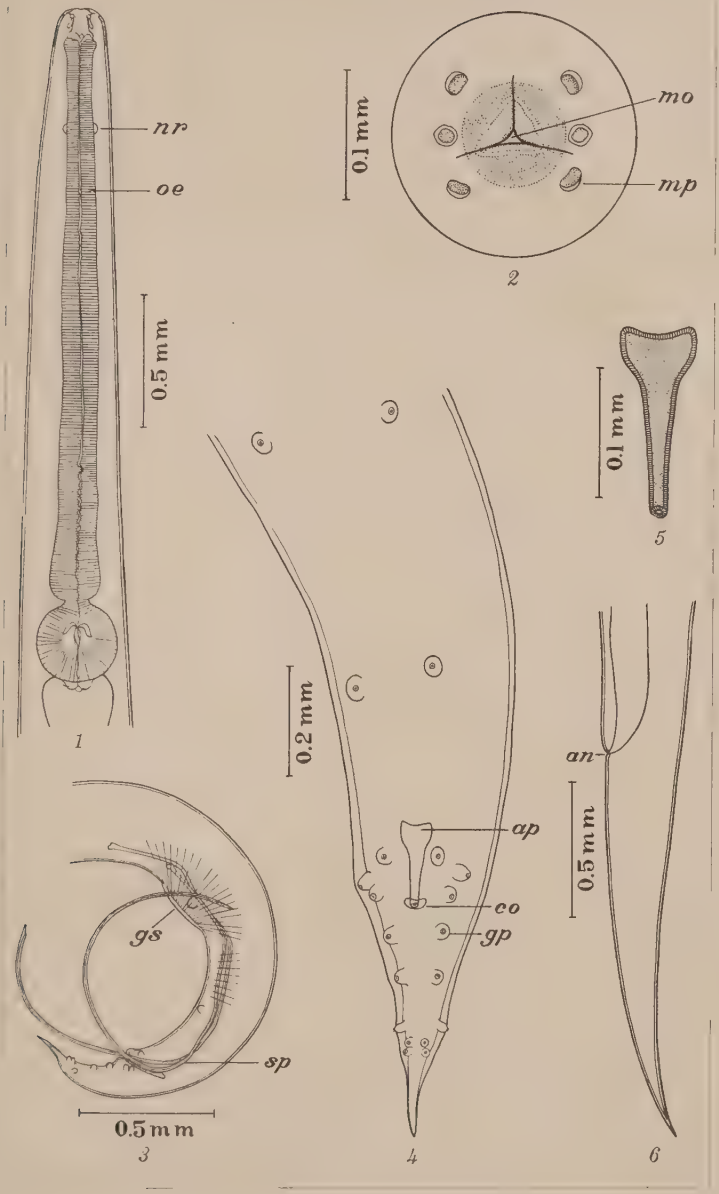
FIG. 1. Anterior end of male, ventral view.
2. Head, seen from its anterior aspect.
3. Posterior end of male, lateral view.
4. Posterior end of male, ventral view.
5. Gubernaculum, ventral view.
6. Posterior end of female, lateral view.

PLATE 2. SPIRONOURA DUYAGI SP. NOV.

FIG. 1. Anterior end of female, ventral view.
2. Head, seen from its anterior aspect.
3. Posterior end of female, lateral view.
4. Posterior end of male, lateral view.
5. Spicules and gubernaculum, ventral view.

PLATE 3. CISSOPHYLLUS LEYTENSIS SP. NOV.

FIG. 1. Anterior end of female, lateral view.
2. Head, seen from its anterior aspect.
3. Posterior end of female, lateral view.
4. Posterior end of male, lateral view.
5. Spicules and gubernaculum, ventral view.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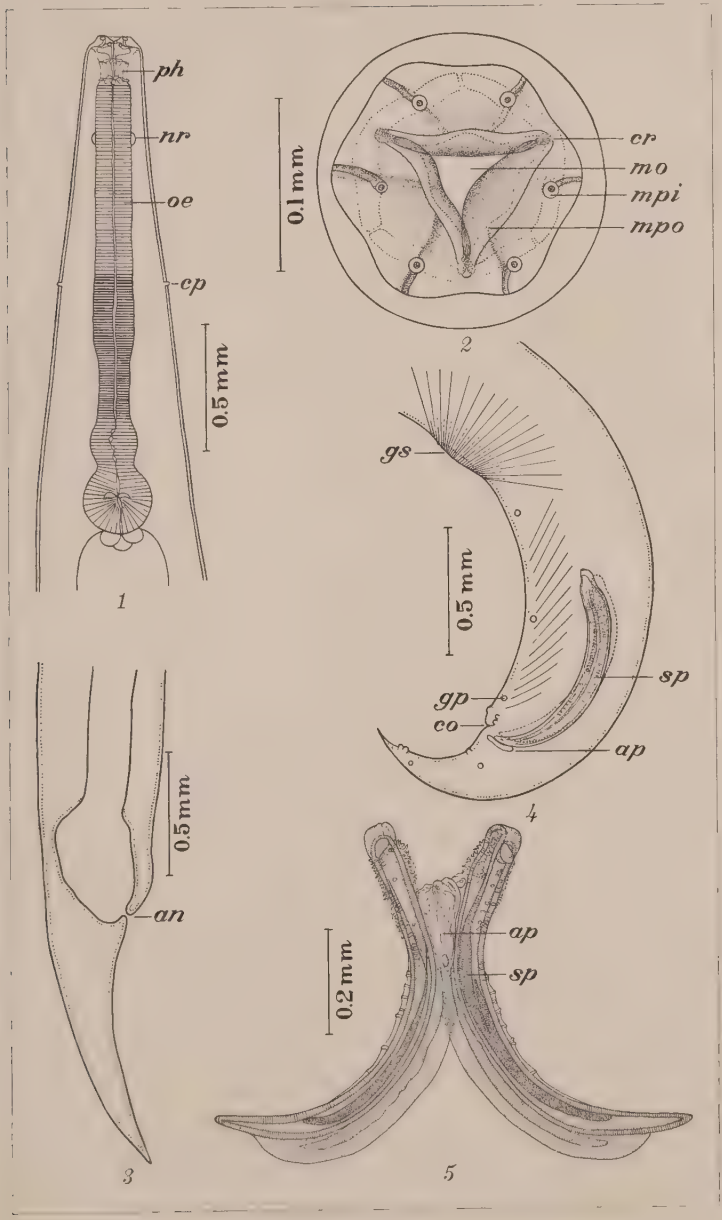
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PLATE 2.

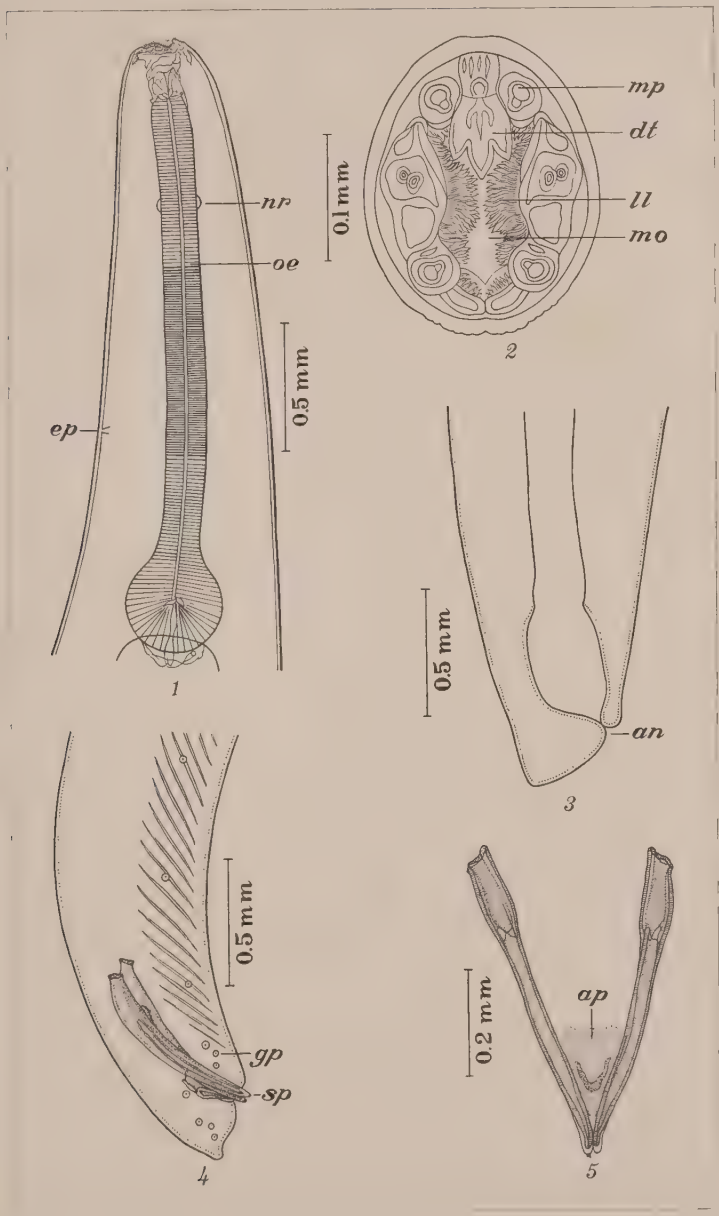

PLATE 3.

GRAM-POSITIVE FORMS OF MYCOBACTERIUM LEPRÆ FROM LEPROTIC LESIONS BACTERIOLOGICALLY NEGATIVE FOR ACID-FAST ORGANISMS: A PRELIMINARY REPORT

By J. Rodriguez, E. Mabalay, and J. C. Tolentino

Of the Bureau of Health, Cebu, Cebu

Much(1) in 1907 devised a number of staining methods in order to determine if the presence of the tubercle bacillus can be demonstrated in such tuberculous lesions as *perlsucht* in cattle and cold abscess in man where it was often impossible to find acid-fast bacilli in smears and yet by culture and pathogenicity experiments the organisms were shown to be present. He brought forth convincing evidence that under certain conditions the bacilli of tuberculosis may be present in the tissues in the form of nonacid-fast granules.

His most successful method was to stain the smear with aniline gentian violet or methyl violet B. N. at 37° C. for twenty-four to forty-eight hours, then to treat it with Lugol's solution, and decolorize it by a mixture of absolute alcohol and clove oil. The decolorization was also accomplished by using successively nitric acid 5 per cent (1 minute), hydrochloric acid 3 per cent (10 seconds), and finally equal parts of acetone and absolute alcohol. In films from miliary tubercles of a calf that had been inoculated with virulent bovine bacilli, he failed to find bacilli when the films were stained by the Ziehl-Neelsen method, but in preparations stained by his own method he found many fine rods, often accompanied by small, rounded granules arranged singly, in pairs, or in short chains resembling beaded bacilli.

Examining the tubercles daily, he found the fine rods and the granules in smears three days after the inoculation, whereas no organism was demonstrated by the Ziehl-Neelsen method till after the sixth day.

Pieces of lung tissue positive by Much's method and showing no acid-fast organism gave rise to fatal generalized tuberculosis in eight weeks when injected subcutaneously in guinea pigs.

Acid-fast bacilli were found in the tissues of the dead animals. Similar results were obtained with tuberculous materials from various sources.

Much concluded that—

1. There is a granular nonacid-fast form of the tubercle bacillus which can be demonstrated by his method.
2. This granular form may be the only stainable form of the tubercle bacillus present in tuberculous organs.
3. The granular form may be accompanied by fine rods which likewise are nonacid-fast.
4. The granular forms are virulent.
5. There are transition forms between the gram-positive granules, the fine gram-positive rods, and the acid-fast rods and granules.

In the magazine containing the report of Much's work is another article on the same subject by Michaélides(2) who, using modifications of the Gram-Nicolle as well as of the Loeffler-Giemsa stains, also found nonacid-fast forms of the tubercle bacillus.

Using either Much's or Michaélides's modifications of the Gram stain, the granules in tuberculosis are seen to be definite, clean cut, round, or oval bodies about 0.5 micron in diameter. Ordinarily, they are stained deep purple, almost black, although there may be a tinge of blue or yellow. They may lie singly or, more frequently, in rows of two to five, or in clusters. There can usually be seen a faint brownish, yellowish, or bluish band connecting the granules, suggesting the body of a bacillus in or on which the granules lie. Unless this band is present, they should not be accepted as true Much's granules.

METHODS

Not being aware of any previous work on the subject, we attempted to demonstrate the presence of nonacid-fast forms of *Mycobacterium lepræ* in leprotic lesions of the skin which were persistently negative for the acid-fast organisms.

After smears were prepared from the cutaneous lesions in the ordinary manner and fixed by heat, Much's modification of the Gram stain was followed, step by step. The stained preparations proved very unsatisfactory at first, and the detection of any Gram-positive organisms corresponding to Much's rods and granules in tuberculosis was rendered almost hopeless, not only on account of the presence of precipitated stains, micrococci,

yeast bodies, and dirt from the surface of the skin, but particularly because of the numerous keratine granules from the granular layer of the epidermis.

In order to minimize this difficulty, smears from lesions showing plenty of acid-fast bacilli were studied first. After fixation with heat, the smears were given a preliminary treatment with solutions of sodium bicarbonate and antiformin in dilutions similar to those employed in preparing sputum for acid-fast examination, after which they were stained according to Much's method. Although this process was rather complicated and many if not most of the organisms were washed away, the preparation became clearer and the chance for error was considerably reduced.

After many trials, the following method was adopted for a time in our routine work:

1. Fix the smear with heat. It is preferable to set the slide aside for four to six hours before staining. If stained too soon, much of the tissue may be washed away in steps 2 and 3.

2. Cover smear with 2 per cent sodium carbonate solution. Duration depends on the thickness of the smear, and is usually five to thirty minutes. Discard the excess.

3. Cover with antiformin and let it stand for five to ten minutes. The antiformin must be fresh. Discard the antiformin and wash. Fix gently with heat again.

4. Cover with one of the following stains: (*a*) Saturated alcoholic solution of methyl violet B. N., 1 part; 2 per cent phenol, 10 parts. (*b*) Saturated alcoholic solution of gentian violet, 1 part; 1 per cent phenol, 9 parts. (*c*) Saturated alcoholic solution of gentian violet, 1 part; saturated aniline water, 3 parts.

5. Discard the excess and add Lugol's solution; let stand for one minute. Wash quickly. Do not blot, as this will likely add dirt and precipitated stain to the preparation.

6. Treat with 5 per cent acid for one minute. Discard the excess; do not wash.

7. Apply 3 per cent hydrochloric acid (HCl) for twenty seconds. Discard the excess.

8. Decolorize by dropping acetone and absolute alcohol (equal parts) on the smear until no more stain is removed.

9. Wash with distilled water.

10. Without drying, counterstain for three to five minutes with Bismark brown.

Although this method has the advantage of being rapid, cheap, and fairly acceptable for routine purposes, much better preparations can be obtained by the use of staining jars in which the slides are placed edgewise. Some recommend that in step 4 the preparation be stained with either aniline gentian violet or methyl violet B. N. for twenty-four to forty-eight hours at 37° C., but in our experience, room temperature is sufficient. This is much more satisfactory than steaming the slide. A little practice is necessary to keep the material in the smear from being completely washed away.

By any of the foregoing methods, *M. lepræ* stains either as a solid rod or more frequently, as a granular bacillus. The granules are definite, cleancut, rounded or oval, with a diameter of about from 0.3 to 0.6 micron, and are deep purple or almost black. There are usually from two to five granules to each organism. Connecting the granules is a brownish or yellowish band suggesting the body of a bacillus in which the granules are embedded.

When smears stained according to the Ziehl-Neelsen method were compared with smears stained by these methods from a leproma known to be rich in acid-fast bacilli, we noted that considerably fewer organisms were demonstrable with the modified Gram stain. The globi were also fewer. This was ascribed to the loss of many bacilli in the preliminary treatment with the sodium carbonate solution and antiformin.

For this important reason, in the latter part of the work, when we had become better acquainted with the appearance of the Gram-positive forms, steps 2 and 3 were omitted and the slide with the fixed smear was stained directly.

Suspecting that both acid-fast and nonacid-fast forms may be present in the same lesion, we have tried various combinations of acid-fast and Gram stains on the same smears. Using Fontes's method, the preparation was stained first with carbol fuchsin, decolorized with acid alcohol, and followed by the ordinary Gram method, counterstaining, however, with Bismark brown. The acid-fast bacilli showed as red or pinkish rods with violet granules, while those which did not fully possess acid-fast properties showed as zigzag violet lines. In our experience, however, many of the acid-fast bacilli are so tinged with violet that it was very difficult to distinguish them from the supposed partially or wholly nonacid-fast bacilli.

We also tried to stain the smear first with carbol fuchsin, then decolorized it with acid, followed by Much's method. All the bacilli took only the Gram stain.

When Much's method was used first, however, and immediately followed by the Ziehl-Neelsen stain, using Bismark brown as a counterstain, it was observed that a large percentage retained the Gram stain. In a few bacilli, we have seen one or two granules which retained the Gram stain while the rest of the granules in the same organisms showed acid-fastness. We took these to be transition forms.

Partially acid-fast and nonacid-fast bacilli may also be demonstrated in ordinary preparations stained according to the Ziehl-Neelsen method. When the staining is carefully done, it will be seen in some smears, particularly those showing many fragmented or granular acid-fast organisms, that some of the bacilli, or granules within the bacilli, are decolorized and take up the counterstain.

In order to avoid possible errors, we have considered in the present report only those Gram-positive nonacid-fast forms in which the granules were arranged in rows—in other words, the bacillary forms. In order to be accepted as true Much's granules the granules had to be connected by the faint brownish or yellowish band. If the single or bunched granules had been included, we should have been able to report a larger number of positives, especially among early cases of leprosy with "closed" lesions. Towards the end of the investigations covered by this report, we became quite confident of being able to distinguish many of these isolated or bunched Much's granules from the other granules in many of our preparations. However, as has been stated, unless the bacillary forms were also present, such cases were reported as negative.

It must be admitted that even with the best technic, the bacilli are always harder to differentiate by the Much method than by the Ziehl-Neelsen stain. Therefore, it needs more than ordinary care to stain by the former method to secure satisfactory results.

In the first place, the slide must be meticulously clean and free from grease spots. The smear must contain as little serum and blood as possible. Oftentimes the success of the method depends on the decolorization; if this is underdone, there will appear numerous extraneous granules, while if it

is carried too far, the granules will not show their typical deep purple or blue-black color.

FINDINGS

Table 1 gives the classification of the patients examined and the percentage found positive with the Ziehl-Neelsen and the Much stains.

TABLE 1.—*Comparative results of the Ziehl-Neelsen and the Much stains.*

Classification of patients.	Examined.	Positive with Z-N stain.		Positive with Much stain.	
		Cases.	Per cent.	Cases.	Per cent.
"Pre-quiescent" (Qt??)	33	15	45.45	19	57.57
"Probationary quiescent" (Qt?)	42	11	26.57	20	47.62
"Candidates for parole"	45	8	17.77	23	51.11
"Quiescents under parole"	48	8	16.66	32	66.66
"Closed" cases of leprosy	78	0	0.00	26	33.33

In order that the significance of these findings may be understood, it is necessary to outline here briefly the regulations of the Bureau of Health governing the examinations which have to be undergone by every candidate for "parole."

As soon as a previously "open" case is declared to be clinically inactive and bacteriologically negative by his treating physician, he is presented before a local examining committee who examine him at least ten times during a period of one year, which has to be spent in segregation. The first four examinations have to be successfully undergone during the first three months, which period is known as the "pre-quiescent period" (abbreviated Qt??). During the succeeding nine months, known as the "probationary period of quiescence" (abbreviated Qt?), at least four examinations have to be made by the local examining committee. In addition, the candidate must be examined at least twice by a National Disposal Committee sent from Manila by the central office of the Bureau of Health. Any candidate found to be bacteriologically positive or to show definite clinical signs of activity in any of these examinations is dropped from the list and has to start from the beginning when he becomes bacteriologically or clinically "negative" again. Eight smears, on an average, are taken at each examination, so that from seventy to eighty smears from each patient have to be found negative consecutively before he can become a can-

didate for parole. At the Eversley Childs Treatment Station, these examinations were made by four different bacteriologists at various stages of the "pre-quiescent" and "probationary quiescent" periods. In spite of these precautions some are always found "positive" at the tenth or last examination before parole.

"Quiescent cases under parole" are required to be examined once every three months for a period of two years, and thereafter, once every six months for a period of four years. As a matter of fact, however, only a small proportion of the cases are examined regularly after parole.

The last visit of the National Disposal Committee to the Eversley Child Treatment Station was made during the second and third weeks of January, 1933. The smears for the Much stain were taken from the same patients examined by the Committee throughout a period extending from about one month previous to and another month after this last visit of the committee.

Eleven of the thirty-three "pre-quiescent" cases were positive and ten were negative to both Ziehl-Neelsen and Much stains. Eight were negative for acid-fast but positive for nonacid-fast bacilli. In four other cases, acid-fast forms were found to be present but were missed by the Much method.

Among the forty-two "probationary quiescents," eleven were found positive by the committee, of whom seven were also positive and three were negative with the Much stain. In one case, acid-fasts were found by the committee in smears from sites not examined with the Much stain. Of the twenty found positive with the Much method, seven were positive also for acid-fasts as already stated, and thirteen, or 30.95 per cent, did not show any organism with the Ziehl-Neelsen stain. Attention is called to the fact that only in a few cases were acid-fast bacilli demonstrable that had been missed by Much's method. The acid-fast forms in such smears were very few.

Eight, or 17.77 per cent, of the forty-five candidates for parole were found positive by the committee and dropped from the list. Twenty-three of the same forty-five patients, or 51.11 per cent, were found positive with the Gram stain. Of the eight cases found by the committee positive for acid-fast bacilli, four were found positive at sites not examined with the Much stain, while in two cases the latter stain failed to show the presence of the organisms demonstrated by the Ziehl-Neelsen method. Two other patients were positive to both stains. Of the twenty-three cases found positive with the Much stain, two were also

found positive for acid-fast bacilli by the committee as already mentioned, five were positive at sites not examined by the committee but examined by us with the Ziehl-Neelsen stain, and fifteen were found negative for acid-fast bacilli at the same sites both by the committee and by ourselves. Therefore, in fifteen of forty-five candidates for parole, or 33.3 per cent, it was possible to show the presence of nonacid-fast bacilli in smears from sites which were found by the committee and by ourselves to be negative for acid-fast forms.

Only forty-eight "quiescent cases under parole" could be examined. Fourteen among them were negative, and six were positive to both the Ziehl-Neelsen and the Much stains. Twenty-six were positive only with the Much method and negative for acid-fast, while but two showed the latter forms of *M. lepræ*, which could not be demonstrated with the Much stain.

It will be noted in Table 1 that while the proportion of those found positive for acid-fasts gradually decreased from the "prequiescents" down to the "quiescents under parole," the proportion found positive with Much's stain does not show such relation.

"Closed" cases of leprosy are patients that show definite clinical manifestations of leprosy, but have never been found bacteriologically positive on routine examinations for acid-fast organisms. Fifty-two of these cases were found negative and twenty-six positive with the Much stain. Of course none of them was found positive for acid-fast forms, otherwise they would have been declared "open" cases. We failed to demonstrate nonacid-fast bacilli in the depigmented macules exhibiting an atrophied appearance of the skin or that showed evidences of returning to normal pigmentation. All macules found harboring the nonacid-fast forms showed clinical evidences of activity such as raised borders or a pink flush, either at the borders, or centrally, or all over the lesion. In not a few of these "closed" cases of leprosy, the nonacid-fast forms were present in fairly large numbers.

Many smears from a number of "leper suspects" and from persons with acute and chronic skin diseases have been examined also with Much's granule stain. Two of these were found positive. One was diagnosed as sarcoid (?) and the other had what appeared to be a chronic eczema on the front of the legs in which leprosy was also suspected.

It must be stated that as a rule only a few nonacid-fast bacilli can be demonstrated by our present methods of staining.

Occasionally, however, such forms were found to be numerous in some of the incipient or "closed" cases of leprosy we have examined.

All of the patients examined with the Much stain will be followed carefully in the future so as to determine whether or not more will become "positive" for acid-fast forms among those found harboring nonacid-fast bacilli than among those who did not show such forms of *M. lepræ*.

REMARKS

While this investigation was in progress, an article by Hoffmann(3) became available to us, but it could not be translated till after the completion of the work covered by this preliminary report. Discussing the granular forms of the leprosy bacillus which he could demonstrate by both the Gram and the Ziehl stains, this investigator came to the conclusion that there were two types of these granules:

1. Degeneration forms showing variability in size and staining properties. These were seen chiefly in improving cases undergoing treatment.

2. Young granules which represented specially resistant forms necessary in the life cycle of the leprosy bacillus. These were found in newly developed lesions and in the blood serum of cases which had never been treated.

Klingmuller(4) showed that granules corresponding to the Much granules in tuberculosis could be demonstrated in leprosy by the careful use of the Ziehl-Neelsen stain and that these granules were capable of producing tissue reaction. Therefore, he concluded that they could not be considered merely as dead particles of the bacillus.

According to the same author, Marchoux was of the opinion that these granular forms were virulent and probably were more resistant forms than the typical bacilli. Paldrock was also quoted as stating that the granules represent reproductive elements.

It is to be noted that none of the workers cited has mentioned Gram-positive forms not demonstrable by the Ziehl-Neelsen stain. So far as we are aware, such forms were first described by Arning and Lweandowsky(5) in an important article which we have read after the completion of our present studies.

Restaining with the Much stain old sections which had been stained previously by either Lustgarten's or Ziehl's methods,

Arning and Lweandowsky obtained the following results in four cases:

First case.—Where formerly no bacilli could be demonstrated, isolated Gram-positive bacilli of the form and size of the leprosy bacillus were found with the Much stain.

Second case.—This case showed exceptionally numerous bacilli, many times the number originally found by Kuhne and Doutrelepont.

Third case.—In an early case of anæsthetic leprosy, Gram-positive forms were found in nerve sections which showed no bacilli by the Ziehl stain.

Fourth case.—In a section from the second case of tuberculoid leprosy reported by Jadassohn, the Much stain showed isolated characteristically formed bacilli within the tuberculoid infiltration. Jadassohn could demonstrate the presence of acid-fast bacilli only with difficulty in this particular case. Repeated and diligent search for acid-fast bacilli in this section by Arning and Lweandowsky gave negative results.

DISCUSSION

The finding of Gram-positive bacilli and granules in lesions of leprosy negative for acid-fasts suggests the possibility that *M. lepræ* may have a Gram-positive, nonacid-fast form or phase as is claimed by some authorities to be the case with *Bacillus tuberculosus*. That such forms are not merely degenerated leprosy bacilli was proved by the fact that they were also found in some of the bacteriologically negative macules of early or "closed" cases of leprosy.

The Gram-positive nonacid-fast forms were seen in smears from the skin in about one-third of the patients with leprotic lesions which were negative for acid-fast organisms, irrespective of whether or not such patients had received treatment, or whether they were "closed" cases or "quiescents" or "negatives" under parole. Therefore, our findings further suggest that the nonacid-fast forms are not affected by the chaulmoogra treatment.

Furthermore, these forms were not seen in the skin in the early anæsthetic, depigmented macule and seldom in pure neural leprosy. Aside from these exceptions, the Gram-positive nonacid-fast bacilli found in the skin did not seem to be limited to any particular stage of the disease.

Our studies with the Much stain in leprosy, correlated with simultaneous examinations with the ordinary Ziehl-Neelsen

stain, lead us to advance the theory that the progress of leprosy in the human body is probably somewhat as follows.

In the very early depigmented macule as well as in the early anæsthetic or maculo-anæsthetic type of leprosy, *M. lepræ* may be present in an as yet undemonstrable form which causes a distinct reaction in the tissues manifested by round-cell infiltration about the capillaries and arterioles. This unrecognized form has a distinct predilection for the cutaneous sensory nerves. It is possible that the organisms at this stage may be so minute as to be ultra-microscopic, or that no staining method has yet been perfected for their demonstration.

It is presumed that as the organisms gradually increase in size, the character of the tissue response also gradually changes until by the time the former are demonstrable by the Much's stain, the pathological picture of the lesion will have become the typical "tuberculoid" arrangement. From the nature of the tissue response, characterized by proliferation of endothelial-like cells, it may be presumed that the main defense of the body during this phase lies in the phagocytic activity of these and similar cells of the reticulo-endothelial system.

Later, due perhaps to the evolution of a necessary step in the life cycle of the invading organism, or to some change in the tissues of the host, the bacilli become acid-fast and the phagocytes are no longer able to dispose of them. Consequently, the bacilli proliferate within the now powerless phagocytic cells to form the well-known "lepra" cells. Using supravital staining methods, Koike(6) has shown these cells to be real histiocytes, following the classification of Sabin. The acid-fast bacilli may be phagocytosed also by the monocytes and polynuclear leucocytes of the blood and are thus distributed to other parts of the body.

When the acid-fast bacilli begin to appear, the lesions become thicker due to accumulation of masses of "lepra cells" and to fibrosis, and also become reddish in color due to new formation of minute blood vessels. New lesions appear at the sites of predilection such as the ear lobes, etc., due to dissemination of the bacilli by the monocytes and polynuclear leucocytes.

At this stage, a variable proportion of the bacilli remain non-acid-fast.

Walker and Sweeney(7) and Schöbl(8) have shown that the chaulmoogra and related oils and their derivatives possess an undoubted specific inhibitory effect in vitro against acid-fast

bacteria such as the tubercle bacillus. This property is not manifested against nonacid-fast microörganisms.

It is possible that chaulmoogra oil may also exercise a similar effect in vivo against the acid-fast stage of *M. lepræ* but not against the nonacid-fast forms. Therefore, the administration of this oil, particularly by the intracutaneous or "plancha," method, where the drug is brought into more or less direct contact with the acid-fast organisms, may bring about their destruction and elimination, together with improvement or disappearance of the infiltrations, nodules, and other manifestations of "activity" of the disease. The patient may then become a "quiescent" or "negative" case, without any manifestations of "activity" and be negative for acid-fasts, but still harbors Gram-positive nonacid-fast bacilli.

When such negatives suffer a "relapse," it simply means that some of the Gram-positive nonacid-fast bacilli have become acid-fast again.

We realize that this hypothesis explaining the development of the leprotic lesions is based on incomplete evidence at the present time. In fact, perhaps this difficult problem will not be solved definitely until after the disease has been successfully transmitted to laboratory animals.

The presence of a possible Gram-positive, nonacid-fast stage in the life cycle of *M. lepræ* is also suggested by the work of Vandremer, Sézary, and Brun,(9) who, using a filtrate of a leprous spleen which passed through a bougie L 3 and which remained apparently sterile in various media for nine months, were able subsequently to obtain Gram-staining pseudomeningococci which later developed into acid-fast bacilli.

CONCLUSIONS

1. By the use of Much's method, it is possible to demonstrate the presence of Gram-positive forms of *M. lepræ* in a considerable percentage of leprotic lesions which do not contain acid-fast bacilli.

2. That many of these nonacid-fast bacilli are not merely degenerated forms may be judged from the fact that they are numerous in many cases of so-called "closed" or "incipient" cases of leprosy which have not undergone treatment.

ACKNOWLEDGMENT

We wish to thank Dr. C. B. Lara, chief physician, Culion Leper Colony, for furnishing us a copy of Hoffmann's article;

and Fr. Clement J. Risacher, S. J., for the translation of the literature.

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A SIMPLE TECHNIC FOR ISOLATING SINGLE TRYPANOSOMES [1]

By TEODULO TOPACIO

Of the Veterinary Research Laboratory, Bureau of Animal Industry Manila

FOUR PLATES

In 1914 Barber,(1) while working in the Bureau of Science, Manila, described a method for isolating single bacteria. His device consisted of a micropipette of such minute dimensions that the point was capable of drawing a single cell from a droplet of bacterial suspension placed in a glass chamber under the microscope. Following this discovery a number of micromanipulators have appeared in literature patterned after Barber's apparatus, among which those of Chambers,(2) Peterfi,(4) and Taylor(5) were the most important. With the rapid progress in cytological research, these appliances have been revised and modified to meet the purpose of each individual investigator. McLung(3) mentions other modifications of lesser importance. It is the concensus of opinion, however, that these devices are not only expensive but likewise complicated, and that it requires considerable training and experience to operate them successfully. In this paper the writer describes a method for isolating single trypanosomes in which, by reason of its simplicity and ease of manipulation, a technician can perform the entire operation after a few preliminary trials. Moreover, the materials that enter into its composition are inexpensive and readily obtainable in any laboratory. The following are the necessary materials of a complete set:

1 Mason jar, 8-ounce capacity, with rubber cork to fit.
1 Pyrex beaker, 250-cc capacity.
12 Oval hollow microscopic slides.
12 Plain microscopic slides.
1 Dispensing bottle, 30-cc capacity.
1 Capillary pipette with rubber nipple.

[1] Bureau of Animal Industry Technical Bulletin 1. From the Veterinary Research Laboratory, Bureau of Animal Industry, Pandacan, Manila. Received for publication June 22, 1933.

1 Test tube, 12 mm by 100 mm, agglutination type.
6 Capillary glass rods, knobbed at both ends, 2 mm by 100 mm.
2 Standard-size Petri dishes, 100 mm by 15 mm.
2 Rubber spatulas, 1 cm sq by 140 mm long, 7 mm by 14 mm.
1 Ordinary glass rod, 130 mm by 5 mm.
1 Surgical knife, bellied blade, 7 mm by 14 cm.
1 Pair cover-glass forceps or section lifter, 120 mm long.
Several pieces of sterilized gauze.
Plasma.
Serum.

These materials are illustrated on Plate 1.

PREPARATION OF MATERIALS

An 8-ounce Mason jar is fitted with a wide rubber cork pierced by a No. 16 brass wire and folded at the lower end to form a suspension hook for the cellophane strips (Plate 1, A). The cellophane cover of an ordinary cigarette box or cigar wrapper is soaked in tap water or alcohol and the outer varnish is removed by rubbing the paper between the fingers. This leaves a thin transparent skeleton paper that is smooth and glossy. The bare paper is washed twelve to twenty-four hours in a beaker of running water as in treating fixed tissues for histological examination. When dry, it is cut into convenient-size strips, usually $\frac{3}{4}$ inch by $2\frac{1}{4}$ inches, which are stored in a clean, wide-mouthed bottle properly stoppered. Sterile serum and plasma are prepared from rabbit blood by making an aseptic heart puncture and collecting the blood in tapered-bottom centrifuge tubes. To collect plasma a 1: 1,000 heparin solution is used, 1 cc to every 10 cc of blood. The charged tubes are centrifuged for ten minutes at 1,800 revolutions per minute, and the plasma is transferred aseptically to a sterile tube. Sterile serum is prepared by slanting the charged tube and allowing the blood to clot overnight in the ice chest. The serum is then transferred into a bottle sterilized for the purpose (Plate 1, D). The entire ensemble of the equipment—glassware, knife, forceps, etc.—is sterilized in an autoclave at 15 pounds pressure for thirty minutes, except the cellophane paper which is sterilized in 85 per cent alcohol for twelve hours in the Mason jar for the purpose (Plate 1, A). The strips are transferred into distilled water in a sterile Petri dish (Plate 1, I) to wash off all traces of alcohol.[2]

[2] Repeated trials showed that cellophane paper in a jar of distilled water could be safely sterilized in the autoclave at 20 pounds steam pressure for half an hour.

Stock suspension of trypanosomes.—With a capillary pipette a drop of infected blood is placed in a hollow slide and a few drops of 30 per cent plasma are added. The mixture is drawn into the pipette and expelled gently several times. A small drop is placed on a slide and examined under the low power of the microscope. If there are found three or four organisms in the drop, the dilution is considered about right.

TECHNIC

A strip of cellophane paper previously sterilized in alcohol is immersed in a Petri dish of sterile distilled water to remove all traces of alcohol. With the cover-glass forceps, it is transferred to a plain sterile slide previously painted with serum fixative (Plate 2, fig. 1). To make a perfectly smooth mount a glass rod made for the purpose is rolled over it (Plate 2, fig. 2). The edges and the surface of the mounted cellophane are wiped perfectly dry with the sterile gauze. The knobbed end of the capillary glass rod is dipped in the suspension of the organisms and quickly touched upon the surface of the paper mount to deposit a tiny drop (Plate 3). This should be done rapidly in order to avoid evaporation. For this purpose, lay the slides carrying the paper mount and the stock suspension side by side in order to perform the act of depositing the drops as rapidly as possible to the point of making it almost instantaneous. After ten separate drops have been deposited 8 mm apart and in two rows, place the slide on the mechanical stage of a microscope and examine the drops under low magnification.[3] The moment that a drop is recognized to contain a single organism, raise the microscope tube, superimpose a tiny drop of 30 per cent plasma and proceed with the examination of the other drops. In this manner it is often possible to spot three or more drops containing single organisms. Each drop with a single organism is removed from the slide by cutting a small square through the paper mount around the drop with a sharp knife as illustrated in Plate 4, fig. 1. The point of the knife is inserted gently beneath the corners of the square to loosen the paper and with the coverglass forceps the section carrying the drop is lifted in toto as in Plate 4, fig. 2. Such preparations may be used for inoculating susceptible animals subcutaneously or intraperitoneally, or for cultivating the organisms in vitro. Needless to say, all used slides, papers, capillary glass rods, etc., should be immersed in the disinfecting solution at the completion of the work.

[3] Ocular 10 x, objective 10 mm, Zeiss microscope.

COMMENT

The advantage of the method just described over the more complicated apparatus of Barber, Chambers, Peterfi, and Taylor is obvious, in so far as the isolation of single trypanosomes is concerned. For such frail organisms as the hæmoflagellates this method of isolation is vastly superior to the pipette method because the delicate structures of the organisms are preserved in the drop, which is not disturbed in the least. Likewise, the transfer of one living organism to a culture medium or to a susceptible animal is absolutely assured without the risk of injuring, destroying, or losing it during the manipulation as might happen when micropipettes are used. Moreover, all the necessary materials for a complete outfit are readily procurable in an ordinary laboratory unit. Above all, are the ease and simplicity of the manipulation, which requires no extraordinary skill. Plasma solution is preferred to other diluents, not only because the trypanosomes survive in it for longer periods, but also because its viscosity is indispensable for making satisfactory minute drops that do not spread out on the paper. If it becomes necessary to perform the manipulations under aseptic conditions, the entire operation can be done under a fumigated glass hood or by the use of a glass chamber especially designed by the author and to be described in another paper.

SUMMARY

A simple technic for isolating single trypanosomes is described and its advantages over the micropipette apparatus are discussed.

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ILLUSTRATIONS

Plate 1. Ensemble of Equipment

A, Mason jar containing 85 per cent alcohol for sterilizing the cellophane paper strips.
B, Beaker containing disinfecting solution 2.5 per cent lysol.
C, Hollow slide for making the dilutions of the parasites in suspension.
D, Sterile serum fixative.
E, Plain microscopic slides for mounting cellophane strips.
F, Capillary pipette for making dilutions.
G, Capillary glass rods with knobbed ends for stirring diluted suspension of protozoans and for depositing tiny drops on the mounted cellophane.
H, Sample of capillary glass rod.
I, Petri dish containing cellophane strips in sterile distilled water ready for use.
J, Flexible rubber spatula for smearing the serum fixative on the slides.
K, Ordinary glass rod, 130 mm by 5 mm, for rolling and mounting the cellophane strips.
L, Surgical knife for cutting sections of cellophane paper carrying the drops of the suspension.
M, Cover-slip forceps for lifting a small section of cellophane paper bearing the single protozoan.

Plate 2

Fig. 1. Mounting the cellophane strips.
2. Rolling the mount to a smooth surface.

Plate 3

Depositing the tiny drops of the suspension with the end of the capillary glass rod.

Plate 4

Fig. 1. Cutting sections through the cellophane paper.
2. Lifting the section of cellophane paper bearing a drop which contains a single organism.

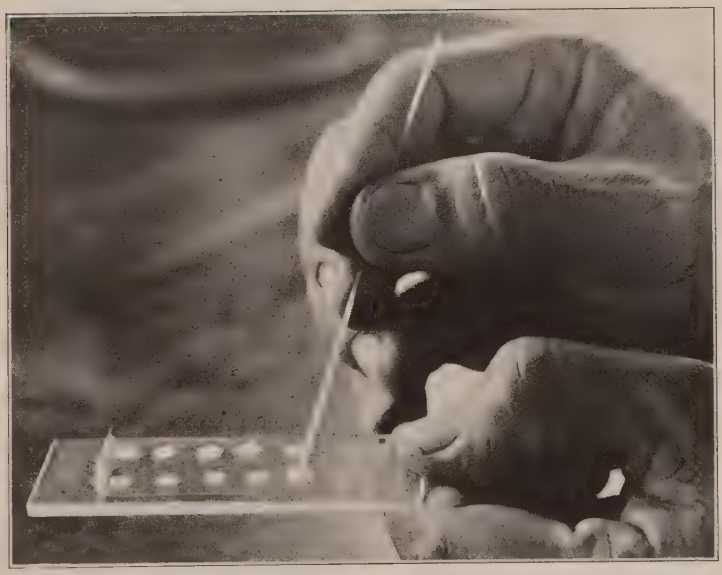
A, Mason jar containing 85 per cent alcohol for sterilizing the cellophane paper strips; B, beaker containing disinfecting solution 2.5 per cent lysol; C, hollow slide for making the dilutions of the parasites in suspension; D, sterile serum fixative; E, plain miscroscopic slides for mounting cellophane strips; F, capillary pipette for making dilutions; G, capillary glass rods with knobbed ends for stirring diluted suspension of protozoans and for depositing tiny drops on the mounted cellophane; H, sample of capillary glass rod; I, Petri dish containing cellophane strips in sterile distilled water ready for use; J, flexible rubber spatula for smearing the serum fixative on the slides; K, ordinary glass rod, 130 mm by 5 mm, for rolling and mounting the cellophane strips; L, surgical knife for cutting sections of cellophane paper carrying the drops of the suspension; M, cover-slip forceps for lifting a small section of cellophane paper bearing the single protozoan.

PLATE 1. ENSEMBLE OF EQUIPMENT.

Fig. 1. Mounting the cellophane strips.

Fig. 2. Rolling the mount to a smooth surface.

PLATE 2.



Depositing the tiny drops of the suspension with the end of the capillary glass rod.

PLATE 3.

Fig. 1. Cutting sections through the cellephane paper.

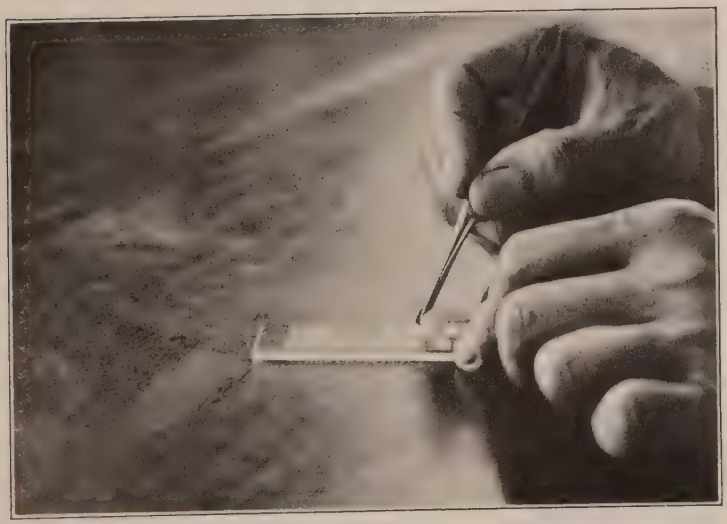


Fig. 2. Lifting the section of cellophane paper bearing a drop which contains a single organism.

PLATE 4.

THE ELECTRIC CHARGE OF RINDERPEST VIRUS [1]

By Teodulo Topacio

Of the Veterinary Research Laboratory, Bureau of Animal Industry Manila

ONE TEXT FIGURE

Not all filters are adapted to the filtration of virus diseases because it is now known that virus particles carry a certain electric charge when exposed to solutions of different hydrogen-ion concentrations. A case in point is the electric charge of foot-and-mouth disease. Olitzky and Boëz(4) found that on the acid of pH 8.0 the virus particles of foot-and-mouth disease carried a positive charge, while in higher alkalinity the charge was negative. Diatomaceous filters—such as, Berkefeld, Mandler, Chamberland, etc.—are generally negatively charged and, if the foot-and-mouth disease virus is to be passed, the concentration of the suspension must fall within pH 8.0 or its alkaline side to avoid being adsorbed or arrested in the candle. Olitzky and Koffman(5) have shown that the particles of tomato mosaic virus behave like mammalian viruses; that is, they carry a negative charge if they are suspended in liquids at pH 5.3 to 8.5; otherwise they carry a positive electric charge. These authors used a cataphoresis cell of the U-tube type and arrived at this conclusion by testing the virus particles recovered from the anode and cathode ends of the tube at varying pH ranges of the suspension under study. Bedson and Bland's(1) blotting-paper method is a much simpler set up and Findlay(3) determined the electric charge of fowl-pox virus and the agent of Rous sarcoma by this same means. He found that fowl-pox virus in suspensions with a pH 6.6 to 8.0 carried a negative charge, while Rous sarcoma also carried a negative charge at pH 5.6 to 8.0. The method is based on the principle that paper carries a negative electric charge and as such all particles in

[1] Bureau of Animal Industry Technical Bulletin 2. From the Veterinary Research Laboratory, Bureau of Animal Industry, Pandacan, Manila. Received for publication June 22, 1933.

suspension having the same charge at certain pH ranges should travel up the paper, and the virus should be present there without being arrested at the point of contact between the paper and the virus suspension. In the following experiments this method of determining electric charge was used.

METHOD AND MATERIALS

Virus.—Mesenteric glands were collected from a rinderpest animal killed at the height of the disease. After trimming the organs free of fat and capsules, a portion was minced with

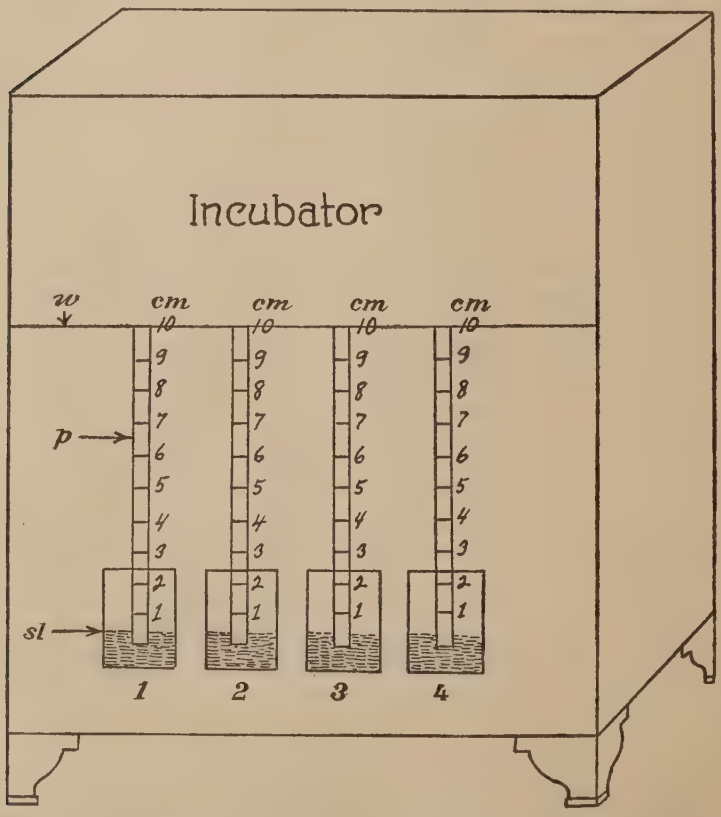


FIG. 1. Author's set up of the Bedson and Bland paper method for determining electric charge; *w*, wire line; *p*, blotting paper; *sl*, suspension level; *1*, *2*, *3*, and *4*, glass vials containing virus suspension in Sorensen's phosphate; *1*, at pH 5.2; *2*, at pH 6.2; *3*, at pH 7.2; *4*, at pH 8.2.

scissors and ground in a sterile mortar. A suspension was prepared in dilution of 1 to 10 with Sorensen's phosphate buffer at pH 5.2, 6.2, 7.2, and 8.2. Ten cubic centimeters of each suspension was placed in the corresponding vials and allowed to extract in a Frigidaire at 9° to 10° C. for one and one-half hours. These constituted the stock virus suspensions used in the experiments. For each experiment a fresh preparation of lymph gland virus was employed.

Paper strips.—White blotting paper was cut in strips 0.5 cm wide and 10 cm long. Each was graduated with lead pencil into 1-cm spaces up to the 9-cm mark, and then sterilized in a dry sterilizer on the day of the test. The charge of the paper was first tested with solutions of eosin and methylene blue to make certain that it carried a negative charge. The result was that the paper used in this experiment was negatively charged, since it arrested the absorption of methylene blue and permitted the eosin to travel through the entire strip.

The test.—A piece of wire was placed across the interior of an incubator heated to a temperature of from 28° to 30° C. The four wide-mouth vials containing the tissue suspensions at pH 5.2, 6.2, 7.2, and 8.2, respectively, were arranged in a straight row directly below the wire (fig. 1). One paper strip was fastened to the wire with a clip or else the top end of the paper was simply folded over the wire to hook it in a vertical position so that the lower end was immersed in the liquid 0.5 cm down. Each vial of the suspension was provided with a strip of paper in this fashion. It is absolutely essential that at no place must the paper touch the sides of the vial to avoid soiling it with the virus material. Absorption was allowed to proceed till the liquid had traveled upward to the 9-cm mark. The time required to accomplish this varied from thirty minutes to one hour.

Bedson and Bland recommend testing the virus from the 2- to the 6-cm mark, using in this case four 1-cm fragments. To abbreviate somewhat the number of tests for each suspension only two segments from centimeters 3 to 4 and 4 to 5 were used in these experiments. After the absorption time had elapsed the segments were macerated separately in 5 cc of corresponding phosphate buffer solutions. The entire suspension of each macerated segment was inoculated subcutaneously into a susceptible animal.

EXPERIMENT 1

Dalupiri carabaos [2] 1169 and 1168 were inoculated subcutaneously with 5 cc of the suspension from centimeters 3 to 4 and 4 to 5, respectively, in phosphate buffer pH 5.2; carabaos 1176 and 1170 received centimeters 3 to 4 and 4 to 5 in phosphate solution pH 6.2; carabaos 1173 and 1175 received centimeters 3 to 4 and 4 to 5 in phosphate solution pH 7.2; and carabaos 1180 and 1174 received centimeters 3 to 4 and 4 to 5 in phosphate solution at pH 8.2 in the order mentioned. To test the virulence of the stock suspensions, 2 cc of each was set aside and was injected separately into susceptible animals which served as controls. Fourteen days after the inoculation of the macerated paper segments, all animals that did not react then received 5 cc each of fresh virulent blood. Table 1 is a summary of results.

TABLE 1.—*Subcutaneous injection of carabaos with paper segments exposed to virus suspensions in Sorensen's phosphate of different pH values.*

Animal No.	pH of virus suspension.	Paper segments suspended in 5 cc Sorensen's solution. Centimeter—	First rise of temperature.	Result.
Carabao.	*pH.*			
1169	5.2	3 to 4	None	No infection.
1168	5.2	4 to 5	do	Do.
1176	6.2	3 to 4	5th day after injection	Killed for rinderpest vaccine.
1170	6.2	4 to 5	do	Do.
1173	7.2	3 to 4	6th day after injection	Do.
1175	7.2	4 to 5	do	Do.
1180	8.2	3 to 4	do	Do.
1174	8.2	4 to 5	7th day after injection	Do.
		CONTROLS		
Cattle.				
1829	5.2	2 cc virus suspension.	3d day after injection	Do.
1819	6.7	do	do	Do.
1972	7.2	do	do	Do.
1977	8.2	do	do	Do.

The result of this experiment was decisive, as might be judged from the reaction following the inoculation of susceptible

[2] A carabao is the native water buffalo.

animals. At pH 5.2 the virus failed entirely to migrate through centimeters 3 to 4 and 4 to 5; consequently, it failed to produce infection. From pH 6.2 to 8.2, however, all the segments injected into the animals produced typical rinderpest, showing that the virus made a perfect migration through the paper. This would indicate that at these pH ranges the virus carried a negative charge. The failure to infect at pH 5.2 would indicate that at this reaction the virus carried a positive charge which enabled the paper to arrest the migration of the virus particles. All animals inoculated with 2 cc of each stock suspension came down with rinderpest, showing that the virus was uniformly infective in the dilution employed.

EXPERIMENT 2

Susceptible native cattle 1678, 1679, 1690, 1910, 1902, 1903, 1113, and 1892 were each injected in the order given with emulsions of paper segments exposed to virus suspensions of varying pH ranges as in experiment 1. Fourteen days after the inoculations, all animals that did not react to the test received 5 cc each of fresh virulent blood. They developed typical rinderpest and were killed for rinderpest vaccine. Table 2 summarizes the result.

TABLE 2.—*Subcutaneous injection of cattle with paper segments exposed to virus suspensions in Sorensen's phosphate of different pH values.*

Animal No.	pH of virus suspension.	Paper segments suspended in 5 cc Sorensen's solution. Centimeter—	First rise of temperature.	Result.
1678	5.2	3 to 4	None	No infection.
1679	5.2	4 to 5	do	Do.
1690	6.2	3 to 4	do	Do.
1910	6.2	4 to 5	6th day after injection	Killed for rinderpest vaccine.
1902	7.2	3 to 4	do	Do.
1903	7.2	4 to 5	5th day after injection	Do.
1113	8.2	3 to 4	do	Do.
1892	8.2	4 to 5	do	Do.

Why segment 3 to 4 failed to infect cattle 1690 is not known. It might be inferred that there was not enough virus in the strip to cause infection in that particular animal. It is also possible that the animal had sufficient resistance to overcome

that dose. The other animals, however, developed rinderpest upon retest with 5 cc of virulent blood, with the exception of animal 1690. Otherwise, the result was an exact replica of the previous experiment on carabaos.

EXPERIMENT 3

This is a repetition of experiment 2 on cattle, using the same technic in every detail. Susceptible cattle 2059, 2066, 2061, 2060, 2063, 2062, 2058, and 2065 were injected in the order recorded in Table 3. Fourteen days after the inoculations all animals that failed to react received 5 cc each of virulent blood. They developed typical rinderpest and were killed for rinderpest vaccine.

TABLE 3.—*Subcutaneous injection of cattle with paper segments exposed to virus suspensions in Sorensen's phosphate of varying pH values.*

Animal No.	pH of virus suspension.	Paper segments suspended in 5 cc Sorensen's solution. Centimeter—	First rise of temperature.	Result.
2059	5.2	3 to 4	None	No infection.
2066	5.2	4 to 5	do	Do.
2061	6.2	3 to 4	do	Do.
2060	6.2	4 to 5	7th day after injection	Temperature reaction recovered.
2063	7.2	3 to 4	do	Killed for rinderpest vaccine.
2062	7.2	4 to 5	5th day after injection	Do.
2058	8.2	3 to 4	do	Do.
2065	8.2	4 to 5	None	No infection.

It is striking that in this experiment segment 3 to 4 exposed to virus suspension at pH 6.2 again failed to infect an animal as in experiment 2. While the individual resistance of the animal might be the principal factor in this particular case, yet it is likely that the behavior of the virus particles themselves in such solution held the greater share of responsibility. Segment 3 to 4 exposed to virus suspension of pH 8.2 also failed to infect. In this instance, however, it is possible that either a certain technical error may have crept in or, again, the particular animal inoculated might have resisted the dose; otherwise it would be difficult to explain why centimeter 4 to 5 of the same strip, being uppermost, should produce a successful infection.

COMMENT

The results of the foregoing experiments indicate that the virus particles of rinderpest are negatively charged when suspended in a solution the hydrogen-ion concentration of which approached the normal blood reaction; namely, pH 7.4. This was shown by the uniform infection produced in cattle and carabaos that received the injection of paper segments exposed to a virus suspension at pH 7.2. When suspended in a solution of pH 6.2, however, the virus particles were found to possess either a positive or negative charge, as was demonstrated by the success or failure to produce infection when susceptible animals were inoculated with the suspension prepared from segments of the same paper strip exposed to a solution of the above-mentioned hydrogen-ion concentration. At pH 5.2 the virus particles appeared to have a positive charge, since in all the experiments the animals injected with papers exposed to virus suspensions of this hydrogen-ion concentration remained unaffected.

The apparent delay in the incubation period in the reacting animals throughout the experiments—namely, first rise of temperature on the fifth to seventh day after inoculation—might be due in a measure to the minute quantity of virus present in the inoculated segments. Again, such factors as the partial adsorption of the virus particles by the paper and the virulence of the virus, not to mention the partial resistance of the animals to the injected dose, collectively might explain such retarded incubation. As demonstrated by Boynton(2) and confirmed by the author, the minimum infective dose of rinderpest blood in this laboratory is 3 cc of a dilution of 1: 10,000. However, animals inoculated with such a dose showed a rise of temperature on the third or fourth day after inoculation.

CONCLUSION

The virus of rinderpest carries a positive charge in a solution of pH 5.2; near the blood range, pH 7.2, the particles uniformly carried a negative charge; while at pH 6.2 they may either be positively or negatively charged, as shown by the success or failure of producing infection with paper segments exposed to a solution of such hydrogen-ion concentration.

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ILLUSTRATION

TEXT FIGURE

FIG. 1. Author's set up of the Bedson and Bland paper method for determining electric charge; *w*, wire line; *p*, blotting paper; *sl*, suspension level; *1*, *2*, *3*, and *4*, glass vials containing virus suspension in Sorensen's phosphate; *1*, at pH 5.2; *2*, at pH 6.2; *3*, at pH 7.2; *4*, at pH 8.2.

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[New names and new combinations are printed in **boldface**.]

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